The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competen with the one chosen by the applicant. The full name or two-letter code of that Authority [redacted] indicated by the applicant on the line below

IPEA/ [redacted]

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION

Applicant's or agent's file reference: 2210130/EJH

International application No.	International filing date *(day/month/year)*	(Earliest) Priority date *(day/month/year)*
PCT/AU99/00705	31 August 1999 (31-08-1999)	31 August 1998 (31-08-1998)

Title of invention

A NOVEL PLANT PROMOTER AND USES THEREFOR

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

THE UNIVERSITY OF QUEENSLAND
St Lucia, Queensland, 4067
Australia

Telephone No.:

Facsimile No.:

Teleprinter No.:

State *(that is, country)* of nationality: Australia

State *(that is, country)* of residence: Australia

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

BOTELLA MESA, Jose Ramon
12 Tad Street
KENMORE, Queensland, 4069
Australia

State *(that is, country)* of nationality: Spain

State *(that is, country)* of residence: Australia

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

CAZZONELLI, Christopher Ian
PO Box 142
MALANDA, Queensland, 4885
Australia

State *(that is, country)* of nationality: Australia

State *(that is, country)* of residence: Australia

☐ Further applicants are indicated on a continuation sheet.

International application No.
PCT/AU99/00705

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [x] agent [ ] common representative

and [x] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

HUGHES, E John L
SLATTERY, John M
CAINE, Michael J

DAVIES COLLISON CAVE
1 Little Collins Street
MELBOURNE VIC 3000
AUSTRALIA

Telephone No.: +61-3-9254 2777

Facsimile No.: +61-3-9254 2770

Teleprinter No.:

[ ] **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination to start on the basis of:

[x] the international application as originally filed

the description
[ ] as originally filed
[ ] as amended under Article 34

the claims
[ ] as originally filed
[ ] as amended under Article 19 (together with any accompanying statement)
[ ] as amended under Article 34

the drawings
[ ] as originally filed
[ ] as amended under Article 34

2. [ ] The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. [ ] The applicant wishes the start of the international preliminary examination to be postponed until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English

[x] which is the language in which the international application was filed.
[ ] which is the language of a translation furnished for the purposes of international search.
[ ] which is the language of publication of the international application.
[ ] which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby elects all eligible States *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes not to elect:

International application No: PCT/AU99/00705

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:		For International Preliminary Examining Authority use only: received	not received
1. translation of international application	sheets	☐	☐
2. amendments under Article 34	sheets	☐	☐
3. copy (or, where required, translation) of amendments under Article 19	sheets	☐	☐
4. copy (or, where required, translation) of statement under Article 19	sheets	☐	☐
5. letter	sheets	☐	☐
6. other *(specify)*	sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. ☐ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ nucleotide and or amino acid sequence listing in computer readable form
6. ☐ other *(specify)*:

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

E John L Hughes
As Agent for the Applicant

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

BRISBANE

PATENT COOPERATION TREATY

WEDNESDAY 19 APR 2000

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: Agent :

DAVIES COLLISON CAVE
1 Little Collins Street
MELBOURNE VIC 3000

PCT

NOTIFICATION OF RECEIPT OF DEMAND BY COMPETENT INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

(PCT Rule 59.3(e) and 61.1(b), first sentence and Administrative Instructions, Section 601(a))

Date of mailing *(day/month/year)*	4 FEB 2000 (4/2/00)
Applicant's or agent's file reference	2210130
	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/AU99/00705	31 AUG 1999 (31/8/99)	31 AUG 1998 (31/8/98)

Applicant

The University of Queensland (et al.)

1. The applicant is hereby **notified** that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

28 JAN 2000 (28/1/00)

2. That date of receipt is:

- [X] the actual date of receipt of the demand by this Authority (Rule 61.1(b)).
- [ ] the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).
- [ ] the date on which this Authority has, in response to the Invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. [ ] **Attention:** That date of receipt is **AFTER** the expiration of 19 months from the priority date. Consequently, the elections(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22). For details, see the *PCT Applicant's Guide, Volume II.*

[ ] *(If applicable)* This notification confirms the information given by telephone, facsimile transmission or in person on:

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/AU **AUSTRALIAN PATENT OFFICE** **PO BOX 200, WODEN ACT 2606, AUSTRALIA** E-mail: pct@ipaustralia.gov.au Facsimile No. 02 6285 3929	Authorized officer MR SEAN MCLACHLAN Telephone No. 02 6283 2357

Form PCT/IPEA/402 (July 1998)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 2210130/EJH	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/AU 99/00705	International filing date *(day/month/year)* 31 August 1999	(Earliest) Priority Date *(day/month/year)* 31 August 1998

Applicant

1. THE UNIVERSITY OF QUEENSLAND et al.

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of **4** sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (See Box II).

4. With regard to the **title**,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   ☐ the text is approved as submitted by the applicant

   ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

   ☐ as suggested by the applicant.

   ☐ because the applicant failed to suggest a figure

   ☐ because this figure better characterizes the invention

   ☒ None of the figures

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 99/00705

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C12N 15/29

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
SEE BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
see below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EMBL:SEQ ID NOS. 1-9
ORBIT (WPAT): C12N - 015/IC and A01H/IC and ACC SYNTHASE OR AMINOCYCLOPROPANE OR ETHYLENE
STN (Medline: dgene) : promoter and gene expression regulation/CT; SEQ. ID. No 2 (inpart).

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,Y	Plant Molecular Biology, Vol. 18, pp 793-797 (1992). Botella et al. See whole document	1-21
X,Y	Plant Molecular Biology, Vol. 20, pp425-436 (1992). Botella et al. p429-p430	1-21
X,Y	Proc. Natl. Acad. Sci. USA, vol. 92, pp1595-1598 (1995) Botella et al. p1597	1-21
P,X	Plant Cell Physiol, 40(4), pp 431-438 (1999). Yoon et al. See whole document	1-21

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" Document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
	27 OCT 1999

Name and mailing address of the ISA/AU

AUSTRALIAN PATENT OFFICE
PO BOX 200
WODEN ACT 2606 AUSTRALIA
E-mail address: pct@ipaustralia.gov.au
Facsimile No.: (02) 6285 3929

Authorized officer

MADHU K. JOGIA
Telephone No.: (02) 6283 2512

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 99/00705

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMBL Accession No. X67100 Liu et al.	1-3, 6, 7
X	Plant Journal, 14(5), pp 573-581. Peck et al. (June 1998) Fig 4; p 577	1-3
X	Plant Molecular Biology, 28(2), pp 293-301 (1995) Peck et al	1-3,8
X	US 5523221 (Weiner , M.P.) published June, 1996. See seq. 1,2 and 3	1-3,8
X	US 5750667 (Wickens et al). published May, 1998 See sequence 7.	1-3, 9
X	US 5756343 (Wu et al) published May, 1998. See sequence 33	1-3, 9
X,Y	WO A 9806852 (University of Hawaii). published 19 Feb 1998	1-21
X,Y	US 5767376 (Stiles et al). published June, 1995.	1-4
X,Y	US 5702933 (Klee et al). published Dec., 1997.	1-4
X	WO A 9814465 (Colorado State University) published April 1998.	1-4
X	US 5723766 (Theologis et al) published June, 1995	1-4
P,X	WO A 9845445 (The Min. of Agriculture et al). published 15.10.98; pp 1-5.	1-21
X	WO A 9711166 (Botella et al) published 27.03.97; p 1-5; claims 1-17	1-21
X	WO A 9635792 (Allrad No. 1 Pty Ltd et al) published 14.11.96. See whole document.	1-21
X	WO A 9727308 (Agritope Inc.et al). published 31.07.91. See whole document.	1-21

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU 99/00705

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
US	5523221						
US	5750667	AU	39597/95	EP	765403	US	5610015
		WO	9629429	US	5677131		
US	5756343	AU	90723/91	CA	2096975	WO	9209617
WO	9806852	AU	40629/97	CZ	9900450	EP	918869
		NO	990508	US	5874269	US	5767376
US	5767376	US	5874269	AU	40629/97	CZ	9900450
		EP	918869	NO	990508	WO	9806852
US	5702933	AU	91137/91	BR	9107191	CA	2096637
		EP	564524	FI	932960	JP	9238689
		NO	923343	WO	9212249	US	5512466
WO	9814465	AU	48929/97	US	5824875		
US	5723766	AU	85114/97	CA	2091243	EP	548164
		MX	9100993	WO	9204456		
WO	9845445	AU	69273/98	ZA	9803007		
WO	9711166	AU	69200/96	EP	854916		
WO	9635792	AU	54930/96	EP	824591		
WO	9727308	AU	17559/97	AU	18466/97	CA	2243850
		CA	2243969	EP	877813	US	5783393
		US	5783394	US	5929302		

END OF ANNEX

WO 00/12714
PCT/AU99/00705

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
HUGHES, E., John, L.
Davies Collison Cave
1 Little Collins Street
Melbourne, VIC 3000
AUSTRALIE

THURSDAY 13 APR 2000

PTO/PCT Rec'd 28 FEB 2001

Date of mailing (day/month/year) 09 March 2000 (09.03.00)	
Applicant's or agent's file reference 2210130/EJH	**IMPORTANT NOTICE**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/AU99/00705	31 August 1999 (31.08.99)	31 August 1998 (31.08.98)

Applicant
THE UNIVERSITY OF QUEENSLAND et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,CN,EP,JP,KP,KR,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CR,CU,CZ,DE,DK,DM,EA,EE,ES,FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MD,MG,MK,MN,MW,MX,NO,NZ,OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,UA,UG,UZ,VN,YU,ZA,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   09 March 2000 (09.03.00) under No. WO 00/12714

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
J. Zahra

Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 2210130	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International application No. PCT/AU99/00705	International filing date *(day/month/year)* 31 August 1999	Priority Date *(day/month/year)* 31 August 1998

International Patent Classification (IPC) or national classification and IPC

Int. Cl. [7] C12N 15/29

Applicant

THE UNIVERSITY OF QUEENSLAND et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheet(s).

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☒	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

Date of submission of the demand	Date of completion of the report
28 January 2000	8 August 2000
Name and mailing address of the IPEA/AU	Authorized Officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	MADHU K. JOGIA Telephone No. (02) 6283 2512

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
[redacted]/AU99/00705

I [redacted] is of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed.

[ ] the description, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[ ] the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages , received on with the letter of

[ ] the drawings, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[ ] the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, was on the basis of the sequence listing:

[ ] contained in the international application in written form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supp rting such statement

1. Statement

Novelty (N)	Claims	YES
	Claims 1-21	NO
Inventive step (IS)	Claims	YES
	Claims 1-21	NO
Industrial applicability (IA)	Claims 1-21	YES
	Claims	NO

2. Citations and explanations (Rule 70.7)

N velty (N) and Inventive Step (IS) Claims 1-21

The notation D1, D2, etc follows the order in which the citations appear in the original International Search Report

The present invention relates to plant promoters wherein the promoter is inducible in response to physical stimulation. More specifically, the promoter directs expression of a gene encoding ACC synthase.

Howev r it appears that the plant promoters are disclosed in the art and that the specific gene sequences are also known. Thus, *Botella et al* (D1, D2 and D3) disclose the ACC synthase gene, including the sequences ID 1-9. Further, sequences 1 and 2 are also disclosed in *EMBL X67100*(D5); Sequence 1 by *Peck et al* (D7); sequence 2 by *Peck et al* (D6); sequence 8 in *US 5523221* (D8) as sequences 1, 2 and 3 and *US 5750667* (D9) as sequence 7; sequence 9 in *US 5756343* (D10) as sequence 33.

Moreover the broader claim 1 is disclosed and taught in *WO 97/27308* (D19) wherein the *dru1* promoter is disclosed. Further, the document teaches the use of the gene which is heterologous to the *dru1* promoter and operably linked to the promoter to enable expression of the product. In addition *Botella* et al (D1) motivates the skilled addressee to further investigate the genes encoding ACC synthase and isolate related nucleotide molecules and determine the response in relation to stress conditions. Similarly, documents D4 and D11-18 disclose and teach plant promoters as defined in claim 1 f your application.

Therefore the invention as defined in claims 1-21 is not novel and lacks an inventive step.

The relevance of the P,X document is discussed in Box VI.

Industrial Applicability Claims 1-21

The invention as defined appears to possess industrial applicability

International application No.
PCT/AU99/00705

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

Application No. Patent No.	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date (valid claim) *(day/month/year)*
P,X WO 9845445	15 October 1998	03 April 1998	09 April 1997

The above document discloses recombinant polynucleotides comprising inducible promoters which include the ACC synthase.

2. Non-written disclosures (Rule 70.9)

Kind of non-written disclosure	Date of non-written disclosure *(day/month/year)*	Date of written disclosure referring to non-written disclosure *(day/month/year)*

International application No.
PCT/AU99/00705

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claim 1 is not fully supported by the description because it broadly defines any isolated nucleic molecule defining a promoter. The specification appears to provide support for a limited number of promoters specifically as defined in claims 4 and 9 for example. Further, it would impose or require an undue burden of experimentation on the part of the skilled addressee to determine exactly which promoters fall within the scope of the claim.

Similarly, claim 15 is not fully supported by the description because the promoter is not fully defined.

PATENT COOPERATION TREATY

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NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

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United States Patent and Trademark Office
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Date of mailing: 09 March 2000 (09.03.00)	
International application No.: PCT/AU99/00705	Applicant's or agent's file reference: 2210130/EJH
International filing date: 31 August 1999 (31.08.99)	Priority date: 31 August 1998 (31.08.98)
Applicant: BOTELLA MESA, Jose Ramon et al	

1. The designated Office is hereby notified of its election made:

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28 January 2000 (28.01.00)

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2210130/EJH

0	For receiving Office use only	
0-1	Int mational Application No.	
0-2	Internati nal Filing Date	
0-3	Name of receiving Office and "PCT International Application"	
0-4	Form - PCT/RO/101 PCT Request	
0-4-1	Prepared using	PCT-EASY Version 2.84 (updated 01.07.1999)
0-5	Petition The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	Receiving Office (specified by the applicant)	Australian Patent Office (RO/AU)
0-7	Applicant's or agent's file reference	2210130/EJH
I	Title of invention	A NOVEL PLANT PROMOTER AND USES THEREFOR
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V	Designation of States	
V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AP: GH GM KE LS MW SD SZ UG ZW and any other State which is a Contracting State of the Harare Protocol and of the PCT EA: AM AZ BY KG KZ MD RU TJ TM and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT EP: AT BE CH&LI CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE and any other State which is a Contracting State of the European Patent Convention and of the PCT OA: BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG and any other State which is a member State of OAPI and a Contracting State of the PCT
V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AE AL AM AT AU AZ BA BB BG BR BY CA CH&LI CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW
V-5	**Precautionary Designation Statement** In addition to the designations made under items V-1, V-2 and V-3, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) of the State(s) indicated under item V-6 below. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit.	
V-6	**Exclusion(s) from precautionary designations**	NONE
VI-1	**Priority claim of earlier national application**	
VI-1-1	Filing date	31 August 1998 (31.08.1998)
VI-1-2	Number	PP5572
VI-1-3	Country	AU
VI-2	**Priority document request** The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s):	VI-1
VII-1	**International Searching Authority Chosen**	Australian Patent Office (ISA/AU)

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VIII	Check list	number of sheets	electronic file(s) attached
VIII-1	Request	4	-
VIII-2	Description (excluding sequence listing part)	63	-
VIII-3	Claims	6	-
VIII-4	Abstract	1	abstract.txt
VIII-5	Drawings	28	-
VIII-6	Sequence listing part of description	9	-
VIII-7	TOTAL	111	
	Accompanying items	**paper document(s) attached**	**electronic file(s) attached**
VIII-8	Fee calculation sheet	✓	-
VIII-15	Nucleotide and/or amino acid sequence listing in computer readable form		-
VIII-16	PCT-EASY diskette	-	diskette
VIII-18	Figure of the drawings which should accompany the abstract	-	
VIII-19	Language of filing of the international application	English	
IX-1	Signature of applicant or agent		
IX-1-1	Name (LAST, First)	HUGHES, E, John, L	

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10-1	Date of actual receipt of the purported international application	
10-2	Drawings:	
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10-2-2	Not received	
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10-4	Date of timely receipt of the required corrections under PCT Article 11(2)	
10-5	International Searching Authority	ISA/AU
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(54) Title: A NOVEL PLANT PROMOTER AND USES THEREFOR

(57) Abstract

The present invention relates generally to a novel plant promoter. More particularly, the present invention provides a plant promoter capable of induction by physical and/or environmental stimuli in cells in which the promoter is indigenous and, in the absence of any negative regulatory mechanism, is capable of constitutive expression in cells in which the promoter is non–indigenous. The present invention is further directed to derivatives of the subject promoter including modular forms of the promoter which are,for example, inducible by different physical and environmental stimuli or which are constitutively expressed. The promoter of the present invention has a range of uses including directing expression of genes conferring useful traits on plants.

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A NOVEL PLANT PROMOTER AND USES THEREFOR

FIELD OF THE INVENTION

The present invention relates generally to a novel plant promoter. More particularly, the present invention provides a plant promoter capable of induction by physical and/or environmental stimuli in cells in which the promoter is indigenous and, in the absence of any negative regulatory mechanism, is capable of constitutive expression in cells in which the promoter is non-indigenous. The present invention is further directed to derivatives of the subject promoter including modular forms of the promoter which are, for example, inducible by different physical and environmental stimuli or which are constitutively expressed. The promoter of the present invention has a range of uses including directing expression of genes conferring useful traits on plants.

BACKGROUND OF THE INVENTION

Bibliographic details of the publications referred to in this specification are collected at the end of the description.

The subject specification contains nucleotide and amino acid sequence information prepared using the programme PatentIn Version 2.0, presented herein after the bibliography. Each nucleotide or amino acid sequence is identified in the sequence listing by the numeric indicator <210> followed by the sequence identifier (e.g. <210>1, <210>2, etc). The length, type of sequence (DNA, protein (PRT), etc) and source organism for each nucl otide or amino acid sequence are indicated by information provided in the numeric indicator fields <211>, <212> and <213>, respectively. Nucleotide and amino acid sequences referred to in the specification are defined by the information provided in num ric indicator fi ld <400> followed by

the sequence identifier (eg. <400>1, <400>2, etc).

The designation of nucleotide residues referred to herein are those recommended by the IUPAC-IUB Biochemical Nomenclature Commission, wherein A represents Adenine, C represents Cytosine, G represents Guanine, T represents thymine, Y represents a pyrimidine residue, R represents a purine residue, M represents Adenine or Cytosine, K represents Guanine or Thymine, S represents Guanine or Cytosine, W represents Adenine or Thymine, H represents a nucleotide other than Guanine, B represents a nucleotide other than Adenine, V represents a nucleotide other than Thymine, D represents a nucleotide other than Cytosine and N represents any nucleotide residue.

The rapidly increasing sophistication of recombinant DNA technology is greatly facilitating research and development of a range of biotechnologically-related industries. This is particularly the case in the horticultural, agricultural and plant industries. Substantial progress, for example, has been achieved in the genetic development of plant varieties exhibiting new or improved traits such as disease resistance, enhanced nutritional properties, greater tolerance to adverse environmental conditions and altered flower colour. However, progress in the genetic manipulation of some plants has been hampered by the lack of sufficient effective promoters and/or the lack of promoters capable of being induced by commercially inexpensive and useful effector stimuli. Furthermore, more promoters are required to facilitate expression of multiple traits in a target species. There is a need, therefore, to identify new promoters and to identify and characterize effector molecules and stimuli which are capable of inducing these promoters. There is also a need to identify promoters which are capable of directing constitutive expression.

Plants are subject to a variety of environmental and mechanical stimuli including stress. Although mechanical stress has been postulated to involve ethylene-mediated meristem morphogenesis (Selker *et al,* 1992), little is known about how mechanical stress induces ethylene production or th signal transduction process

involved.

In work leading to the present invention, the inventors sought to identify and isolate promoters involved in mechanical stress-induced expression of genetic traits in *Vigna radiata* (mung bean). Mung bean plants are a useful model for physical and chemical induction of phenotypic expression of genetic traits due to their morphology, rapid growth rate and the ability to obtain a large number of uniform plants and, therefore, sufficient amounts of tissues to conduct analyses.

In accordance with the present invention, the inventors have isolated a promoter capable of induction following physical stimulus in cells in which the promoter is indigenous, i.e. cells of mung bean plants. The promoter is also capable of being induced by a range of chemical and other environmental stimuli. However, in cells in which the promoter is non-indigenous, the promoter is constitutively expressed. The promoter of the present invention is useful in the genetic manipulation of plants.

SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

The promoter of the present invention is referred to herein as "pGEL-1". The promoter was referred to as the "AIM-1 promoter" (or in some cases *"AIM-1"*) in the priority application. Reference herein to *"AIM-1"* means the structural gene encoding ACC synthase from *Vigna radiata.*

One aspect of the present invention provides an isolated nucleic acid molecule

comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein, in its native form, the promoter is inducible in response to physical stimulation.

Another aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof, wherein said promoter, in its native form, directs expression of a gene associated with ethylene production and is inducible by physical stimulation.

Yet another aspect of the present invention relates to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter, in its native form, directs expression of a gene encoding ACC synthase and is inducible by physical stimulation.

Still another aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter, in its native form, directs expression of a gene encoding ACC synthase or another gene associated with ethylene biosynthesis and is inducible by physical stimulation.

Still yet another aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter, in its native form, directs expression of a gene encoding an ACC synthase having an amino acid sequence substantially as set forth in <400>2 or an amino acid sequence having at least 60% similarity to <400>2.

A further aspect of the present inv ntion relates to an isolated nucleic acid molecule

comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter, in its native form, directs expression of a gene encoding ACC synthase and wherein said gene comprises a nucleotide sequence substantially as set forth in <400>1 or a sequence having at least 50% similarity thereto and/or a nucleotide sequence capable of hybridizing to <400>1 under low stringency conditions.

Still another aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter, in its native form, comprises a nucleotide sequence substantially as set forth in <400>3 or a nucleotide sequence having at least 25% similarity thereto or a nucleotide sequence capable of hybridizing to <400>3 under low stringency conditions.

Another aspect of the present invention provides a modular promoter, said modular promoter comprising at least one portion which is derived from a promoter which, in its native form, directs expression of a gene associated with ethylene biosynthesis and is inducible by physical stimulation.

Yet another aspect of the present invention is directed to a modular promoter, said modular promoter comprising at least one portion which is derived from a promoter which, in its native form, directs synthesis of an ACC synthase having an amino acid sequence substantially as set forth in <400>2 or an amino acid sequence having at least 60% similarity thereto.

Still yet another aspect of the present invention is directed to a modular promoter, said modular promoter comprising at least one portion which is derived from a promoter which, it is native form, directs synthesis of an ACC synthase encoded by a gene comprising a nucleotide sequence substantially as set forth in <400>1 or a nucleotide sequence having at least 50% similarity ther to or a nucleotide

sequence capable of hybridizing to <400>1 under low stringency conditions.

In still yet another aspect of the present invention, there is provided a modular promoter comprising a portion which is derived from a promoter which comprises, in its native form, a nucleotide sequence substantially as set forth in <400>3 or a nucleotide sequence having at least 25% similarity thereto or a nucleotide sequence capable of hybridizing to <400>3 under low stringency conditions.

Another aspect of the present invention contemplates a genetic construct comprising a promoter or modular promoter each as herein defined or a derivative or homologue thereof, means to facilitate insertion of a nucleotide sequence downstream of and operably linked to said promoter and optionally a gene encoding a selectable marker.

A further aspect of the present invention provides a genetic construct comprising a promoter or modular promoter each as herein defined or a derivative or homologue thereof, a nucleotide sequence operably linked to said promoter and optionally a gene encoding a selectable marker.

Another aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof which is capable of constitutive expression in cells in which the promoter is non-indigenous.

Yet another aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof, wherein said promoter, in its native form, directs expression in response to physical stimulation of a gene associated with ethylene production and which promoter in a non-native host cell is constitutively expressed.

Still yet another aspect of the present invention relates to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter, in its native form, directs expression in response to physical stimulation of a gene encoding ACC synthase and which promoter in a non-native host cell is constitutively expressed.

A further aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter, in its native form, directs expression of a gene encoding ACC synthase or another gene associated with ethylene biosynthesis and in a cell in which the promoter is indigenous, the promoter is inducible by physical stimulation whereas in a cell in which the promoter is non-indigenous, the promoter is constitutively expressed.

Another aspect of the present invention provides an isolated an isolated acid molecule comprising a sequence of nucleotides or complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof, wherein, in its native form, the promoter is inducible in response to physical stimulation and wherein the promoter is selected from the list consisting of:

(i) a promoter which, in its native form, directs expression of a nucleotide sequence substantially as set forth in <400>1;

(ii) a promoter which, in its native form, directs expression of a nucleotide sequence which hybridizes under low stringency conditions to <400>1;

(iii) a promoter which, in its native form, directs expression of a nucleotide sequence having at least about 50% similarity to <400>1;

(iv) a promoter which, in its native form, directs expression of a nucleotide

sequence which encodes an amino acid sequence substantially as set forth in <400>2;

(v) a promoter which, in its native form, directs expression of a nucleotide sequence which encodes an amino acid sequence which has at least about 60% similarity to <400>2;

(vi) a promoter comprising a nucleotide sequence substantially as set forth in <400>3;

(vii) a promoter comprising a nucleotide sequence capable of hybridizing to <400>3 under low stringency conditions; and

(viii) a promoter comprising a nucleotide sequence having at least about 25% similarity to <400>3.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a representation of the oligonucleotide primers used in Long Distance Inverse PCR.

Figure 2 is a diagrammatic representation showing generation of *Spe*I and *Xba*I fragments of pGEL-1.

Figure 3 is a diagrammatic representation of pGEL-1 sequencing strategy.

Figure 4 is a representation of the nucleotide sequence of pGEL-1 (2470 bp).

Figur 5 is a diagrammatic representation of th construction of full length pGEL-1.

Figure 6A(i) - 6A(xii) are diagrammatic representations of plasmids pPZP2.5GuNt,

pPZP2.5LuNt, pPZP1.4GuNt, pPZP1.4LuNt, pPZP35SGuNt, pPZP35SLuNt, pPZP017GuNt, pPZP023GuNt, pPZP045GuNt, pPZP070GuNt, pPZP088GuNt and pPZP1.1GuNt, respectively. Gu, GUS; Lu, luciferase (LUC); Nt, Nos terminator; 35S, cauliflower mosaic virus 35S promoter. The number given after the term "pPZP" represents the length of the promoter sequence in kilobases. For example, pPZP2.5LuNt contains the full length promoter, pGEL-1.

Figure 6B is a diagrammatic representation of the backbone vector pPZP111 (Hajdukiewicz *et al,* 1994).

Figure 6C is a diagrammatic representation of the vector pGuNt.

Figure 7 are photographic representations showing (A) and (B) transgenic tobacco lines containing pGEL-1:GUS gene assayed to visualise GUS activity; and (C) wild-type tobacco stained for GUS (negative control).

Figures 8(a) and (b) are graphical representations showing GUS activity in young tobacco plants, transformed with pGEL-1:GUS and CaMV35S:GUS constructs. 2.5G#3-4 and 2.5G#7-3 are two independent transgenic lines containing full length promoter, pGEL-1, fused to the GUS gene; 35SG#5-2 is a transgenic line containing CaMV35S promoter fused to the GUS gene. (A) is GUS activity measured as nmoles Mu per minute per mg protein. (B) is GUS activity measured as nmoles Mu per minute per gram fresh weight (gfw) of pant material. Mu is equal to 4-methyl-umbrelliferone.

Figure 9 is a graphical representation showing quantitative analysis of pGEL-1 and 35S cauliflower mosaic virus (CaMV) promoter: GUS fusions in mature vegetative transgenic tobacco. (A) expressed as nmoles of Mu produced per minute per mg protein; (B) expressed as nmoles of Mu produced per minute per gram fresh weight (gfw) of plant material.

Figure 10 is a graphical representation showing a quantitative analysis of pGEL1 and 35SCaMV promoter:GUS fusions in mature flowering transgenic tobacco. (A) expressed as nmoles of Mu produced per minute per mg protein; (B) expressed as nmoles of Mu produced per minute per gram fresh weight (gfw) of plant material.

Figure 11 is a graphical representation showing quantitative analysis of a range of deletions of pGEL-1:GUS fusions in mature vegetative transgenic tobacco. Deletions range from 1.027 bp to 86 bp. Activity is expressed as nmoles of Mu produced per minute per gram fresh weight (gfw) of plant material.

Figure 12 is a diagrammatic representation showing deletions in pGEL-1.

Figure 13 is a photographic representation of Southern analysis of three T2 homozygous independent tobacco transgenic lines (3-4, 7-3 and 10-3) containing pGEL-1 fused to the GUS gene, and one T2 homozygous tobacco transgenic line (5-2) containing the CaMV 35S promoter fused to the GUS gene. Genomic DNA was digested with *Eco*RI (E) or *Bam*HI (B) restriction enzymes. A ^{32}P-labelled DNA fragment containing the full GUS gene and Nos terminator was used as a probe. Lane 1 contained size markers. Lanes 2 and 3: line 3-4; lanes 4 and 5: line 7-3; lanes 6 and 7: line 10-3; lane 8: line 5-2.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is predicated in part on the identification of a promoter directing expression of a gene. The gene encodes 1-aminocyclopropane-1-carboxylic acid synthase ("ACC synthase") and is inducible, in its native form, by physical stimuli (Botella *et al,* 1992; Botella *et al,* 1995). Reference herein to "native form" with respect to a promoter means the promoter in cells in which the promoter is normally resident, i.e. indigenous. In the present case, cells from mung bean plants are cells in which the promoter is indigenous. When the promoter is transferred by genetic means to non-mung bean plant cells, the resulting cells are an example of cells carrying a non-indigenous promoter.

Accordingly, the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein, in its native form, the promoter is inducible in response to physical stimulation.

More particularly, the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof, wherein said promoter, in its native form, directs expression of a gene associated with ethylene production and is inducible by physical stimulation.

Even more particularly, the present invention relates to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter, in its native form, directs expression of a gene encoding ACC synthase and is inducible by physical stimulation.

In a related embodiment, the present invention relates to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of

nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter, in its native form, directs expression of a gene encoding ACC synthase or another gene associated with ethylene biosynthesis and is inducible by physical stimulation.

Although the present invention is exemplified by the identification and isolation of the promoter directing synthesis of ACC synthase from *Vigna radiata* (mung bean), the present invention extends to any promoter which, in its native form, is inducible in response to physical stimulation and which directs expression of a nucleotide sequence having at least about 50% similarity to the nucleotide sequence set forth in <400>1 and/or nucleotide sequence capable of hybridizing to the nucleotide sequence of <400>1 under low stringency conditions, such as at 42°C.

Examples of promoters contemplated by the present invention include but are not limited to promoters directing expression of genes associated with ethylene biosynthesis such as the gene encoding ACC synthase.

The gene encoding ACC synthase from mung bean is referred to as *AIM-1*. ACC synthase from mung bean comprises the amino acid sequence substantially as set forth in <400>2.

Accordingly, another aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter, in its native form, directs expression of a gene encoding an ACC synthase having an amino acid sequence substantially as set forth in <400>2 or an amino acid sequence having at least 60% similarity to <400>2.

The percentage similarity at the amino acid or nucleotide sequence level is generally to a portion comprising at least about 20 contiguous amino acids or at least about 60 contiguous nucleotide bases. Preferably, however, the comparison

is made to the entire amino acid sequence or entire nucleotide sequence. Alternative percentage similarities include at least about 70%, at least about 80%, at least about 90% and at least about 95% or above or discrete percentages there between.

Genes encoding ACC synthase enzymes not having 100% similarity to <400>2 include derivatives and homologous of the mung bean enzyme. A derivative includes parts, fragments, mutants and fusions of the mung bean ACC synthase defined in <400>2 including ACC synthase enzymes having one or more amino acid substitutions, additions and/or deletions to the amino acid sequence of <400>2. Homologues include enzymes from closely or distantly related plants including fungi.

A particularly preferred promoter of the present invention directs expression of *AIM-1*. The nucleotide sequence of *AIM-1* is set forth in <400>1.

According to this embodiment, there is provided an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter in its native form directs expression of a gene encoding ACC synthase and wherein said gene comprises a nucleotide sequence substantially as set forth in <400>1 or a sequence having at least 50% similarity thereto and/or a nucleotide sequence capable of hybridizing to <400>1 under low stringency conditions, such as at 42°C.

For the purposes of defining the level of stringency, those skilled in the art will be aware that several different hybridization conditions may be employed. For example, a low stringency may comprise a hybridization and/or a wash carried out in 6xSSC buffer, 0.1% w/v SDS at from about room temperature to about 44°C such as from about 28 C to about 42°C or equivalent condition sufficient for annealing of primers in a polymeras chain reaction or hybridization of oligonucleotide to DNA or RNA. A medium stringency may comprise a

hybridization and/or wash carried out in 2xSSC buffer, 0.1% w/v SDS at a temperature in the range of from about 45°C to about 65°C. A high stringency may comprise a hybridization and/or wash carried out in 0.1xSSC buffer, 0.1% w/v SDS at a temperature of at least about 65°C. The buffers may also contain from 0% to about 10 to about 15% v/v formamide for use in the hybridization and/or washing solutions.

Generally, the stringency is increased by reducing the concentration of SSC buffer, and/or increasing the concentration of SDS in the hybridization buffer or wash buffer and/or increasing the temperature at which the hybridization and/or wash are performed. Conditions for hybridizations and washes are well understood by one normally skilled in the art. For the purposes of clarification of parameters affecting hybridization between nucleic acid molecules, reference can conveniently be made to pages 2.10.8 to 2.10.16 of Ausubel *et al* (1987), which is herein incorporated by reference.

Alternative percentage similarities include those set forth above.

Nucleotide sequences not having 100% similarity to <400>1 include derivatives and homologues of mung bean *AIM-1*. A derivative includes, parts, fragments, mutants and fusions of the mung bean *AIM-1* defined in <400>1 including *AIM-1* genes having one or more nucleotide substitutions, additions and/or deletions to the nucleotide sequence of <400>1. Homologues include genes from closely or distantly related plants including fungi.

The term "similarity" as used herein includes exact identity between compared sequences at the nucleotide or amino acid level. Where there is non-identity at the nucleotide level, "similarity" includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. Wher there is non-identity at the amino acid level, "similarity" includes amino acids that are nevertheless related

to each other at the structural, functional, biochemical and/or conformational levels. In a particularly preferred embodiment, nucleotide and sequence comparisons are made at the level of identity rather than similarity. Any number of programs are available to compare nucleotide and amino acid sequences. Preferred programs have regard to an appropriate alignment. One such program is Gap which considers all possible alignment and gap positions and creates an alignment with the largest number of matched bases and the fewest gaps. Gap uses the alignment method of Needleman and Wunsch (1970). Gap reads a scoring matrix that contains values for every possible GCG symbol match. GAP is available on ANGIS (Australian National Genomic Information Service) at website http://mel1.angis.org.au.

Most preferably, the promoter of the present invention comprises a nucleotide sequence substantially as set forth in <400>3 or a functional derivative or homologue thereof.

Accordingly, another aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter in its native form comprises a nucleotide sequence substantially as set forth in <400>3 or a nucleotide sequence having at least 25% similarity thereto or a nucleotide sequence capable of hybridizing to <400>3 under low stringency conditions such as at 42°C.

Still another aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides or complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof, wherein, in its native form, the promoter is inducible in response to physical stimulation and wh rein the promoter is selected from the list consisting of:

(i) a promoter which, in its native form, directs expression of a nucleotide

sequence substantially as set forth in <400>1;

(ii) a promoter which, in its native form, directs expression of a nucleotide sequence which hybridizes under low stringency conditions to <400>1;

(iii) a promoter which, in its native form, directs expression of a nucleotide sequence having at least about 50% similarity to <400>1;

(iv) a promoter which, in its native form, directs expression of a nucleotide sequence which encodes an amino acid sequence substantially as set forth in <400>2;

(v) a promoter which, in its native form, directs expression of a nucleotide sequence which encodes an amino acid sequence which has at least about 60% similarity to <400>2;

(vi) a promoter comprising a nucleotide sequence substantially as set forth in <400>3;

(vii) a promoter comprising a nucleotide sequence capable of hybridizing to <400>3 under low stringency conditions; and

(viii) a promoter comprising a nucleotide sequence having at least about 25% similarity to <400>3.

The determination of low stringency conditions may be done from about room temperature to about 44°C. Preferably, low stringency is determined at 28°C. Alternatively, low stringency is determined at 42°C.

The promoter of the present invention is useful in the development of genetic constructs to xpress heterologous nucleotide sequences placed downstream of,

and operably linked to, the promoter.

Reference herein to a "promoter" is to be taken in its broadest context and includes the transcriptional regulatory sequences of a classical genomic gene, including the TATA box which is required for accurate transcription initiation, with or without a CCAAT box sequence and additional regulatory elements (i.e. upstream activating sequences, enhancers and silencers) which alter gene expression in response to developmental and/or environmental stimuli, or in a tissue-specific or cell-type-specific manner. A promoter is usually, but not necessarily, positioned upstream of or 5′ to a structural gene, the expression of which it regulates. Furthermore, the regulatory elements comprising a promoter are usually positioned within 2 kb of the start site of transcription of the gene.

In the present context, the term "promoter" is also used to describe a synthetic or fusion molecule, or derivative which confers, activates or enhances expression of a structural gene or other nucleic acid molecule in a plant cell.

The term "operably in connection" or "operably linked to" in the present context means placing a structural gene under the regulatory control of the promoter of the present invention by positioning the structural gene such that the expression of the gene is controlled by the promoter. Promoters and the like are generally positioned 5′ (upstream) to the genes that they control. In the construction of heterologous promoter/structural gene combinations, it is generally preferred to position the promoter at a distance from the gene transcription start site that is approximately the same as the distance between that genetic sequence or promoter and the gene it controls in its natural setting, i.e., the gene from which the promoter is derived. As is known in the art, some variation in this distance can be accommodated without loss of function.

As used herein, a "structural gene" shall be taken to refer to that portion of a gene comprising a DNA segment encoding a protein, polypeptide or a portion thereof or

alt rnatively, an isolated nucleic acid molecule which does not necessarily encode a polypeptide, such as an antisense, ribozyme, abzyme or co-suppression molecule.

The term "structural gene" also refers to copies of a structural gene naturally found within the cell, but artificially introduced, or the structural gene may encode a protein not normally found in the plant cell into which the gene is introduced, in which case it is termed a heterologous gene. A heterologous structural gene may be derived in whole or in part from a bacterial genome or episome, eukaryotic genomic or plastid DNA, cDNA, viral DNA, or chemically synthesized DNA. It is possible that a structural gene may contain one or more modifications in either the coding or the untranslated regions which affect the biological activity or the chemical structure of the expression product, the rate of expression, or the manner of expression control. Such modifications include, but are not limited to, mutations, insertions, deletions, and substitutions of one or more nucleotides.

Where the structural gene encodes a polypeptide, it may constitute an uninterrupted coding sequence or it may include one or more introns, bounded by the appropriate plant-functional splice junctions. The structural gene may be a composite of segments derived from a plurality of sources, naturally occurring or synthetic. The structural gene may also encode a fusion protein, as long as the experimental manipulations maintain functionality in the joining of the coding sequences.

Another aspect of the invention relates to the use of the promoter of the present invention or a derivative or homologue or modular form thereof in the identification and/or isolation of similar promoter sequences associated with from other genes.

According to this embodiment, there is contemplated a method for identifying a r lated nucleic acid molecule which is at least capable of conferring, increasing or otherwise facilitating the expression of a structural gene, when in native form, in

response to physical stimulation, said method comprising contacting genomic DNA or parts or fragments thereof, with a hybridization-effective amount of the nucleotide sequence set forth in <400>1 or <400>3, or a part, analogue or derivative thereof or a complementary sequence thereto, and then detecting said hybridization.

Another aspect of the present invention contemplates a nucleic acid molecule defining a promoter or a homologue or derivative thereof said nucleic acid molecule obtainable by the method of isolating genomic DNA from plant cells, rendering the genomic DNA or portion thereof single stranded and then identifying a region on genomic DNA which hybridizes to a primer corresponding to all or part of <400>1 or a complementary form thereof and then cloning DNA upstream of the region of primer hybridization.

The related genetic sequence may be in a recombinant form, in a virus particle, bacteriophage particle, yeast cell, animal cell, or a plant cell. Preferably, the related genetic sequence originates from an agriculturally-important or horticulturally-important plant such as potato, tomato, wheat, barley, canola, oats, maize, sugar cane, cotton or rice and/or wild varieties and/or hybrids or derivatives and/or ancestral progenitors of same. Horticulturally important plants include rose, carnation, petunia, lisianthus, lily, iris, tulip, freesia, delphinium, limonium, pelargonium as well as fruit and vegetable crops such as papaya.

The present invention clearly extends to an isolated nucleic acid molecule which comprises a sequence of nucleotides which overlaps with the sequence set forth in <400>1 or <400>3.

Preferably, such isolated nucleic acid molecules comprise genomic DNA which is isolated using polymerase chain reaction or hybridization approaches based upon the nucleotide information disclosed in <400>1 or <400>3.

Preferably, the genetic sequence set forth in <400>1 or <400>3, or a derivative or

analogue th reof, is labelled with a reporter molecule capable of producing an identifiable signal (e.g. a radioisotope such as ^{32}P, or ^{35}S, or a biotinylated molecule) to facilitate its use as a hybridization probe in the isolation of related nucleic acid molecules.

An alternative method contemplated in the present invention involves hybridising a nucleic acid primer molecule of at least 10 nucleotides in length, derived from <400>1 or <400>3, or a derivative or analogue thereof, to a nucleic acid "template molecule", said template molecule herein defined as for example, genomic DNA, or a functional part thereof. Specific nucleic acid molecule copies of the template molecule are amplified enzymatically in a polymerase chain reaction, a technique that is well known to one skilled in the art.

Preferably, the nucleic acid primer molecule or molecule effective in hybridization is contained in an aqueous mixture of other nucleic acid primer molecules. More preferably, the nucleic acid primer molecule is in a substantially pure form.

The nucleic acid template molecule may be in a recombinant form, in a virus particle, bacteriophage particle, yeast cell, animal cell, or a plant cell. Preferably, the related genetic sequence originates from an agricultural or horticultural plant or other suitable plant species.

The present invention extends to the subject promoter in a genetic construct.

The term "genetic construct" is used in its broadest sense to include an isolated nucleic acid molecule comprising a sequence of nucleotides.

The genetic construct is conveniently engineered so as to include means to facilitate insertion of a nucleotide s quence in a region 3′ of the promoter, to place a nucleotide sequence downstream of and operably linked to, the promoter which then directs its transcription. Such a "means" includes but is not limited to a

restriction endonuclease-mediated insertion, homologous recombination, transposon insertion, PCR mediated insertion and random insertion. Preferably, the means is a restriction endonuclease site. Generally, the inserted restriction site is unique to the genetic construct or may be represented, for example, twice but separated by a nucleic acid sequence which is deleted upon restriction digestion of the genetic construct. The required nucleotide sequence to be transcribed is then inserted into the deleted region.

The genetic construct of the present invention may comprise solely the promoter and optionally a nucleotide sequence downstream thereof or, alternatively, may comprise additional nucleotide sequences constituting promoter regulatory region(s), transcribed sequence regulatory regions, a marker (eg. antibiotic resistance, chemical compound resistance or enzyme such as β-galactosidase (GUS) or luciferase (LUC) β-glucuronidase), autonomous replication region and/or genome integration sequence. The promoter may be the naturally occurring promoter or may be an active fragment or part thereof or a derivative, analogue or homologue of the promoter.

Accordingly, another aspect of the present invention contemplates a genetic construct comprising a promoter or modular promoter each as herein defined or a derivative or homologue thereof, means to facilitate insertion of a nucleotide sequence operably linked to said promoter and optionally a gene encoding a selectable marker.

More particularly, this aspect provides a genetic construct comprising a promoter or modular promoter as herein defined or a derivative or homologue thereof, one or more unique restriction sites down stream of said promoter to enable the insertion of a heterologous nucleotide sequence operably linked to said promoter and a gene encoding a selectable marker.

In a related embodiment, the present invention provides a genetic construct

comprising a promoter or modular promoter each as herein defined or a derivative or homologue thereof, a nucleotide sequence operably linked to said promoter and optionally a gene encoding a selectable marker.

The present invention extends to genetic constructs in which the genetic sequence of the invention, or a functional derivative, part, fragment, homologue, or analogue thereof, is operably linked to a structural gene sequence. The invention is not, however, limited by the nature of the structural gene sequence contained in such genetic constructs.

In one embodiment, the structural gene sequence is a reporter gene, such as but not limited to the β-glucuronidase gene, or the chloramphenicol acetyl transferase gene, or the firefly luciferase gene, amongst others.

In an alternative embodiment, the structural gene sequence encodes, or is complementary to a structural gene sequence encoding, a cytotoxin or other gene product which, when produced in a plant cell, kills or significantly alters host cell metabolism to limit cell division.

In a further alternative embodiment, the structural gene sequence encodes, or is complementary to a structural gene sequence encoding, a hormone polypeptide or polypeptide which is involved in the biosynthesis of a hormone or other molecule. The invention particularly contemplates the expression of a phytohormone molecule under control of the promoter defined in <400>3 or an analogue or derivative thereof, to produce a high local concentration of said phytohormone in the undifferentiated cells which is sufficient to result in the development of a floral meristem or vegetative meristem, depending upon the nature of the phytohormone.

In a still further alternative embodiment, th structural gene sequence may be a ribozyme, abzyme, antisense or co-suppression molecule which targets the expression of a gene. According to this embodiment, expression of such a

structural gene under the control of the g netic sequence of the invention will partially or completely reduce, delay or inhibit the expression of said structural gene.

Yet another alternative embodiment comprises a structural gene whose product facilitates the accumulation of a molecule which itself or a further metabolic or oxidised form thereof facilitates a change in the colour of plant tissue, cells, organs, leaves or flowers. For example, the structural gene may encode a flavonoid pathway enzyme or a cytochrome P450 molecule such as a plant, mammalian or bacterial monooxygenase.

Wherein the structural gene being targeted is normally expressed in more than one cell type, the expression of said structural gene under control of the promoter of the present invention may further result in the gene being expressed in a cell-type or tissue-type specific pattern.

The genetic construct according to this aspect of the invention may further comprise a transcription termination sequence, placed operably in connection with the structural gene sequence.

In an alternative embodiment, the transcription termination sequence is placed downstream of the promoter of the present invention, optionally spaced therefrom by a nucleotide sequence which comprises one or more restriction endonuclease recognition sites, to facilitate the insertion of a structural gene sequence as hereinbefore defined between said genetic sequence and said transcription termination sequence.

The term "terminator" refers to a DNA sequence at the end of a transcriptional unit which signals termination of transcription. Terminators are 3'-non-translated DNA sequences containing a polyadenylation signal, which facilitates the addition of polyadenylate sequences to the 3'-end of a primary transcript. T rminators active

in cells derived from viruses, yeasts, moulds, bacteria, insects, birds, mammals and plants are known and described in the literature. They may be isolated from bacteria, fungi, viruses, animals and/or plants.

Examples of terminators particularly suitable for use in the genetic constructs of the present invention include the nopaline synthase (NOS) gene terminator of *Agrobacterium tumefaciens,* the terminator of the Cauliflower mosaic virus (CaMV) 35S gene, the *zein* gene terminator from *Zea mays*, the Rubisco small subunit (SSU) gene terminator sequences, subclover stunt virus (SCSV) gene sequence terminators, any *rho*-independent *E. coli* terminator, amongst others.

The genetic construct of the instant invention may further include an origin of replication sequence which is required for replication in a specific cell type, for example a bacterial cell, when said genetic construct is required to be maintained as an episomal genetic element (eg. plasmid or cosmid molecule) in said cell.

Preferred origins of replication include, but are not limited to, the *f1*-ori and *col*E1 origins of replication.

In a further alternative embodiment, the genetic construct of the invention further comprises one or more selectable marker genes or reporter gene sequences, placed operably in connection with a suitable promoter sequence which is operable in a plant cell and optionally further comprising a transcription termination sequence placed downstream of said selectable marker gene or reporter gene sequences.

As used herein, the term "selectable marker gene" includes any gene which confers a phenotype on a cell in which it is expressed to facilitate the identification and/or selection of cells which are transfected or transformed with a genetic construct of th invention or a derivativ thereof.

Suitable selectabl mark r genes contemplated herein include the ampicillin

resistance gene (Amp^r), tetracycline resistance gene (Tc^r), bacterial kanamycin resistance gene (Kan^r), phosphinothricin resistance gene, neomycin phosphotransferase gene (*npt*II), hygromycin resistance gene, β-glucuronidase (GUS) gene, chloramphenicol acetyltransferase (CAT) gene and luciferase gene, amongst others.

Those skilled in the art will be aware that the choice of promoter for expressing a selectable marker gene or reporter gene sequence may vary depending upon the level of expression required and/or the species from which the host cell is derived and/or the tissue-specificity or development-specificity of expression which is required.

Examples of promoters suitable for use in expressing selectable marker or reporter gene in the genetic constructs of the present invention include promoters derived from the genes of viruses, yeasts, moulds, bacteria, insects, birds, mammals and plants which are capable of functioning in isolated plant cells or whole organisms regenerated therefrom, including whole plants. The promoter may regulate the expression of the selectable marker gene or reporter gene constitutively, or differentially with respect to the tissue in which expression occurs, or with respect to the developmental stage at which expression occurs, or in response to external stimuli such as physiological stresses, pathogens, or metal ions, amongst others.

Examples of promoters include the CaMV 35S promoter, NOS promoter, octopine synthase (OCS) promoter, *Arabidopsis thaliana SSU* gene promoter, napin seed-specific promoter, P_{32} promoter, BK5-T *imm* promoter, *lac* promoter, *tac* promoter, phage lambda λ_L λ_R or promoters, CMV promoter (U.S. Patent No. 5,168,062), T7 promoter, lacUV5 promoter, SV40 early promoter (U.S. Patent No. 5,118,627), SV40 late promoter (U.S. Patent No. 5,118,627), adenovirus promoter, baculovirus P10 or polyhedrin promoter (U.S. Patent Nos. 5,243,041; 5,242,687; 5,266,317; 4,745,051; and 5,169,784), and the like. In addition to the specific promoters identified herein, cellular promoters for so-called housekeeping genes are useful.

Those skilled in the art will b aware of additional promoter sequences and terminator sequences which may be suitable for use in performing the invention. Such sequences may readily be used without any undue experimentation.

A still further embodiment contemplates a genetic construct which further comprises one or more integration sequences.

As used herein, the term "integration sequence" shall be taken to refer to a nucleotide sequence which facilitates the integration into plant genomic DNA of a genetic sequence of the invention with optional other integers referred to herein.

Particularly preferred integration sequences according to this embodiment include the left border (LB) and right border (RB) sequences of T-DNA derived from the Ti plasmid of *Agrobacterium tumefaciens* or a functional equivalent thereof.

Another aspect of the invention provides a method of expressing a structural gene in a plant cell, said method comprising introducing into said plant cell a genetic construct comprising a promoter sequence which is at least capable of conferring, increasing or otherwise regulating expression of a structural gene to which it is operably connected in a plant cell, wherein said promoter sequence preferably comprises the nucleotide sequence set forth in <400> 3, or a functional derivative, part, fragment, homologue, or analogue thereof which is at least 25% similar thereto or a complementary sequence thereto or a sequence capable of hybridising to <400>3 under low stringency conditions such as 28°C or 42°C and wherein said structural gene is operably linked to said promoter sequence on said genetic construct.

The method according to this aspect of the invention is particularly useful for the xpr ssion of a wide range of foreign structural genes in cells of plants, including a cell cycle control protein; an antibody-expressing gene, such as a SCAB gene; a selectable marker gene that confers resistance against kanamycin,

phosphinothricin, spectinomycin or hygromycin, amongst others; a reporter gen including GUS, CAT, LUC and pigment genes, amongst others; a gene encoding a regulatory protein which modulates expression of a gene in plant cells; and a gene which encodes a developmental regulatory protein, such as, for example, a homeobox protein, that is involved in regulating the developmental fate of a cell. As will be apparent from the disclosure herein, the present method is clearly applicable to the expression of antisense molecules, ribozyme molecules, co-suppression molecules, gene-targeting molecules, or other molecules that are intended to modulate the expression of one or more endogenous plant genes.

A further aspect of the present invention provides a transfected or transformed cell, tissue, organ or whole organism which comprises the promoter or its derivatives or homologues of the present invention. Preferably, the cell, tissue, organ or whole organism expresses a structural gene operably under the control of said promoter sequence.

This aspect of the invention clearly encompasses a transgenic plant such as a crop plant or flowering plant, transformed with a recombinant DNA molecule which comprises at least a genetic sequence which is at least 25% similar to <400>3.

The genetic construct of the present invention may be introduced into a cell by various techniques known to those skilled in the art. The technique used may vary depending on the known successful techniques for that particular organism.

Means for introducing recombinant DNA into bacterial cells, yeast cells, or plant, insect, fungal (including mould), avian or mammalian tissue or cells include, but are not limited to, transformation using $CaCl_2$ and variations thereof, direct DNA uptake into protoplasts, PEG-mediated uptake to protoplasts microparticle bombardment, lectroporation, microinjection of DNA, microparticle bombardment of tissue explants or cells, vacuum-infiltration of tissue with nucleic acid, or in the case of plants, T-DNA-mediated transfer from *Agrobacterium* to the plant tissu .

For microparticle bombardment of cells, a microparticle is propelled into a cell to produce a transformed cell. Any suitable ballistic cell transformation methodology and apparatus can be used in performing the present invention. Exemplary apparatus and procedures are disclosed by Stomp *et al.* (U.S. Patent No. 5,122,466) and Sanford and Wolf (U.S. Patent No. 4,945,050). When using ballistic transformation procedures, the genetic construct may incorporate a plasmid capable of replicating in the cell to be transformed.

Examples of microparticles suitable for use in such systems include 0.1 to 10 μm gold or tungsten spheres such as a 0.5-5 μm gold or tungsten sphere. The DNA construct may be deposited on the microparticle by any suitable technique, such as by precipitation.

Once introduced into the plant tissue, the expression of a structural gene under control of the promoter of the present invention may be assayed in a transient expression system, or it may be determined after selection for stable integration within the plant genome.

Where the cell is derived from a multicellular organism and where relevant technology is available, a whole organism may be regenerated from the transformed cell, in accordance with procedures well known in the art.

Those skilled in the art will be aware of the methods for transforming, regenerating and propagating other type of cells, such as those of fungi.

In the case of plants, plant tissue capable of subsequent clonal propagation, whether by organogenesis or embryogenesis, may be transformed with a genetic construct of the present invention and a whole plant regenerated therefrom. The particular tissue chosen will vary depending on the clonal propagation systems available for, and best suit d to, the particular species being transformed. Exemplary tissue targets include leaf disks, pollen, embryos, cotyl dons,

hypocotyls, megagametophytes, callus tissue, existing m ristematic tissue (e.g., apical meristem, axillary buds, and root meristems), and induced meristem tissue (e.g., cotyledon meristem and hypocotyl meristem).

The regenerated transformed plants may be propagated by a variety of means, such as by clonal propagation or classical breeding techniques. For example, a first generation (or T1) transformed plant may be selfed to give homozygous second generation (or T2) transformants, and the T2 plants further propagated through classical breeding techniques.

The regenerated transformed cells contemplated herein may take a variety of forms. For example, they may be chimeras of transformed cells and non-transformed cells; clonal transformants (e.g., all cells transformed to contain the expression cassette); grafts of transformed and untransformed tissues (e.g., in plants, a transformed root stock grafted to an untransformed scion).

The promoter of the present invention, in its native form (i.e. in cells in which it is indigenous), is inducible by physical stimulus which includes mechanical stress, movement, vibration, air pressure, water stress and the like. Other non-mechanical stimuli also induce the instant promoter including auxins, abscisic acid, salt concentration amongst others. Non-mechanical stimuli include environmental stimuli such as but not limited to chemical induction of the promoter. The promoter may also be developmentally regulated and/or may be tissue or organ specific.

As stated above, the identification of a promoter capable of induction by physical or mechanical stimuli provides a particularly useful basis for developing a range of genetically altered plants. For example, air movement may be used to activate expression of a nucleotide sequence operably linked to the subject promoter. This may be useful during the commercial cultivation of large numbers of plants. Generating air movement such as generated by fanning, or a change in air pressure over and/or around the plants can be used to activat expression of the

promoter. Alternatively, or in addition, water droplets generated mechanically or by controlling humidity may be used to stimulate promoter activity. Heterologous nucleotide sequences operably linked to the promoter are then expressed. Such heterologous sequences may encode, for example, resistance to insect or other pathogens, salt tolerance, enzymes which manipulate the flow of metabolites down particular biochemical pathways, enzymes which alter the nutritional content of certain types of plant tissues including seeds and other reproductive parts and antisense, co-suppression, ribozyme or deoxyribozyme molecules to down regulate expression of an endogenous gene. Examples of the latter would be to render a plant male or female sterile, to alter biochemical pathways or to otherwise alter the characteristics of the target plant, such as to inhibit ethylene biosynthesis or to delay senescence.

Accordingly, another aspect of the present invention contemplates a method of altering a characteristic of a plant said method comprising introducing a genetic construct into a cell or group of cells of a plant, said genetic construct comprising a promoter as herein defined and a nucleotide sequence operably linked to said promoter and wherein said nucleotide sequence facilitates the altering of said plant characteristic, regenerating a plant or plantlet from said cell or group of cells carrying said genetic construct and growing or subjecting said plant or plantlet to conditions sufficient to induce the promoter in said genetic construct.

The genetically altered plant may be subjected to physical stimulus such as mechanical stress in order to induce the promoter. Alternative forms of stimulus, however, are also contemplated by the subject invention such as water droplets, air movement, air pressure and chemical stimuli such as auxins. The promoter may also be constitutively expressed.

An altered characteristic may be readily determined by comparing a transgenic plant with a non-transgenic plant of the same sp cies. The comparison may be at the biochemical, physiological or visual l vel. Altered characteristics include but are

not limited to resistance to plant viruses, bacteria, fungi, nematodes and other pathogens, improved nutritional value (eg. using sunflower high sulphur gene), an expression of an "antibody" (often referred to as a "plantabody"), altered biochemical pathways, altered fertility, altered flower colour amongst many other characteristics.

The promoter of the present invention is in its native form, inducible by a range of stimuli including physical, environmental, chemical and genetic. The promoter comprises, therefore, different regulatory areas for different stimuli. The present invention contemplates the manipulation of the subject promoter such that it is inducible by a particular stimulus or stimuli.

Accordingly, another aspect of the present invention provides a modular promoter, said modular promoter comprising at least one portion which is derived from a promoter which, in its native form, directs expression of a gene associated with ethylene biosynthesis and is inducible by physical stimulation.

More particularly, the present invention is directed to a modular promoter, said modular promoter comprising at least one portion which is derived from a promoter which, in its native form, directs synthesis of an ACC synthase having an amino acid sequence substantially as set forth in <400>2 or an amino acid sequence having at least 60% similarity thereto.

Even more particularly, the present invention is directed to a modular promoter, said modular promoter comprising at least one portion which is derived from a promoter which, it is native form, directs synthesis of an ACC synthase encoded by a gene comprising a nucleotide sequence substantially as set forth in <400>1 or a nucleotide sequence having at least 50% similarity thereto or a nucleotide sequence capable of hybridizing to <400>1 under low stringency conditions.

Still more particularly, the present invention provides a modular promot r

comprising a portion which is derived from a promoter which comprises, in its native form, a nucleotide sequence substantially as set forth in <400>3 or a nucleotide sequence having at least 25% similarity thereto or a nucleotide sequence capable of hybridizing to <400>3 under low stringency conditions.

Low stringency may be determined at about from room temperature to about 44 C such as at 28°C to 42°C (e.g. 28°C or 42°C).

A "modular" promoter is considered as an example of a "derivative". Another derivative contemplated by the present invention includes the deletion of negatively acting *cis* element(s). This aspect of the present invention is predicated on the observation of high expression of the promoter in the presence of the protein synthesis inhibitor, cycloheximide, which inhibits production of a highly unstable, short-lived negative regulator (transcription factor) of the subject promoter. Accordingly, by deleting the negative *cis* element(s), higher inducible or even constitutive expression of the promoter may be obtained.

Another aspect of the present invention contemplates an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof which is capable of constitutive expression in cells in which the promoter is non-indigenous.

This aspect of the present invention is predicated on the surprising observation that the promoter of the present invention, when placed in plant cells in which it is not indigenous, i.e. non-mung bean cells, is constitutively expressed. Although not intending to limit the present invention to any one theory or mode of action, it is proposed that in cells in which the promoter is indigenous, a negative regulatory molecule prevents constitutive expression of the promoter. This negative regulatory molecule would not normally be present in other plant cells and, henc , the promoter is constitutively expressed.

Accordingly, another aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof, wherein said promoter, in its native form, directs expression in response to physical stimulation of a gene associated with ethylene production and in which in a non-native host cell is constitutively expressed.

More particularly, a further aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter, in its native form, directs expression of a gene encoding ACC synthase or another gene associated with ethylene biosynthesis and in a cell in which the promoter is indigenous, the promoter is inducible by physical stimulation whereas in a cell in which the promoter is non-indigenous, the promoter is constitutively expressed.

The present invention further contemplates a transgenic plant carrying the promoter of the present invention or parts, limbs, flowers, petals, reproductive portions or seeds thereof or progeny or clones thereof.

The present invention is further described by the following non-limiting Examples.

EXAMPLE 1

Detection of mechanical strain-induced gene

A gene encoding 1-aminocyclopropane-1-carboxylic acid synthase ("ACC synthase"), induced *inter alia* by mechanical strain, auxin and salt stress was isolated according to Botella *et al* (1992;1995). The cDNA sequence and corresponding amino acid sequence is shown in <400>1. The amino acid sequence alone is shown in <400>2. This gene is referred to herein as *AIM-1*. Its promoter is referred to herein as "pGEL-1".

EXAMPLE 2

Cloning of the ACC Synthase gene (*AIM-1*) promoter (pGEL-1)

(a) Recirculation of DNA

Ten micrograms of genomic DNA isolated by CsCl purification was digested with 2.5 U/μg of *Hind*III in the presence of 0.1 M spermidine, extracted with 1 volume phenol:chloroform:isamyl alcohol (25:24:1) and precipitated by addition of 0.1 vol NaOAc and 2 volumes EtOH. DNA was then re-ligated with 9 Weiss units of T4 DNA ligase and purified using Bresatec's Bresa Clean Kit. The effectiveness of recircularisation was analyzed by gel electrophoresis.

(b) Long Distance Inverse Polymerase Chain Reaction (LDIPCR) procedure

A reaction mixture of 2 mM $MgSO_4$ pH 9.1, containing 60 mM Tris-SO_4 and a small number, e.g. see $MgSO_4$, 18 mM $(NH_4)_2$ SO_4, 0.2 mM of each dNTP, 0.2 μM of NSE oligonucleotide primers (see Figure 1), sterile water and 300 ng of recircularised

genomic DNA was prepared in a total volume of 40 μl. The reaction mixture was vortexed and briefly spun prior to incubation at 94°C to prevent non-specific primer interactions. Before initialising the thermal cycle, 10 μl of sterile water containing 1 μl of Life Technologies' eLONGase enzyme mix (TaqI/Vent polymerases) was added to the reaction and mixed by pipetting. An equal volume of mineral oil was layered over the mix to prevent evaporation. The optimised PCR parameters are shown in Table 1.

TABLE 1

PCR profile times and temperatures used during amplification and reamplification protocols.

Optimised Temperatures and Times				
Amplification	Initial Step	Denaturation	Anneal and Extension	
	60 sec. 94°C	30 sec. 94°C	480 sec. 68°C	
		45 cycles		
Reamplification	Initial Step	Denaturation	Anneal	Extension
	60 sec. 94°C	30 sec. 94°C	30 sec. 62°C	480 sec. 68°C
		35 cycles		

After the final step of thermal cycling, 1 volume of chloroform-isoamyl alcohol (24:1) was added to remove the oil layer and the samples were stored at 4°C.

Cloning Strategy

The circularised genomic DNA was first amplified with oligonucleotide primers NSE-1 and NSE-2 (refer to Figure 1). The products of this first amplification were further reamplified using either NSE-3/NSE-4 or NSE-5/NSE-6 (Figure 1). Electrophoretic

analysis of the amplification products, generated with both combinations of primers, revealed a DNA fragment of approximately 4 kb.

EXAMPLE 3

Analysis of 4 kb fragment

The 4 kb product obtained with NSE3/NSE-4 was excised from the gel and purified with glassmilk (Bresatec's Bresa Clean). As attempts at cloning the 4kb product were initially unsuccessful, alternative strategies were devised. The purified 4 kb product was digested with *Xba*I and two fragments of 1.3 kb and 0.9 kb (see Figure 2) were sub-cloned into the vector pGEM11 (Promega corporation, USA), which had been previously digested with *Xba*I giving the plasmids pGX1.3 and pGX0.9, respectively. The 4 kb fragment was also digested with *Spe*I and blunt-ended before cloning the digestion products into pGEM11 (previously digested with *Xho*I and blunt-ended). As a result, two *Spe*I fragments of 1.1 kb and 1.4 kb (see Figure 2) were sub-cloned and the plasmids named pGS1.1 and pGS1.4, respectively. The 1.4 kb fragment did not show any *Spe*I recognition sequences in one of its ends, indicating that some exonuclease activity had taken place during the blunt-ending process.

EXAMPLE 4

Reconstruction and sequencing of the 2.5kb pGEL-1 region

The sequencing strategy for pGEL-1 is shown in Figure 3. Sequencing was performed using the dideoxy chain termination method (Sanger *et al,* 1977) using a Applied Biosystems kit (Applied Biosystems, USA). Analysis of the sequences revealed that the four clones partially overlapped. The 1.3 kb *Xba*I and 1.1 kb *Spe*I fragments contained the 5' untranslated region of the *AIM-1* cDNA, confirming that this region is upstream of the *AIM-1* gene. As a result, a partial restriction map for a 2.5 kb region of the 4 kb DNA fragment was generated. The nucleotide sequence of pGEL-1 is shown in Figure 4 and in <400>3.

With this information in hand, the promoter region was reconstructed by the following strategy (refer to Figure 5). pGS1.4 was digested with *Hind*III and blunt ended. The promoter insert was then excised with *Spe*I, obtaining a 1.4 kb fragment with blunt-*Spe*I ends (see Figure 5(a)).

pGS1.1 was linearised with *Sal*I and blunt ended. Later the linearised construct was digested with *Spe*I resulting in a linearised vector with blunt-*Spe*I ends containing the 3' end of the promoter region (Figure 5(b)). The fragment excised in (a) was ligated into (b) to reconstruct the 2.5 kb pGEL-1 promoter (Figure 5(b)).

EXAMPLE 5

Characterization of pGEL-1

(a) Generation of deletion fragments and chimeric gene constructs

To fully characterize pGEL-1, two different lengths of the promoter sequence were used: the entire 2.5 kb sequence and a 1.4 kb fragment upstream of the first ATG codon. β-Glucuronidase *(GUS)* and luciferase (*LUC*) reporter genes were each ligated to one or other of the promoter fragments and to the 3' terminator region from the *Agrobacterium tumefaciens* nopaline synthase gene (NOS) to generate a series of chimeric gene constructs.

A series of 7 deletions in the promoter region were also generated, starting from 170 base-pairs upstream of the first ATG codon. Each of these was likewise ligated to the NOS 3' terminator region and to the *GUS* reporter gene. Intermediate vectors containing each of the promoter fragments (0.17, 0.23, 0.45, 0.70, 0.88, 1.1, 1.4, 1.6 or 2.5 kb) ligated to the *GUS* reporter gene and NOS terminator were generated in pBluescript. Intermediate vectors comprising the promoter fragments 1.4 and 2.5 kb were also ligated to the *LUC* reporter gene with the NOS terminator. For control purposes, additional constructs containing the cauliflower mosaic virus 35S promoter linked to either *GUS* or *LUC* were also prepared.

These chimeric constructs were then successfully ligated into the polylinker of the binary vector backbone pPZP111 (Hajdukiewicz *et al,* 1994), for use in plant transformation. A range of these constructs, comprising pGEL-1 is shown in Figures 6A(i) to 6A(xii). The backbone vector pPZP111 is shown in Figure 6B. The bluescript vector comprising *GUS* and the NOS terminator (pGuNt) is shown in Figure 6C.

(b) Transformation and regeneration of tobacco

The characterization of pGEL-1 was carried out using tobacco as the model plant system. Tobacco transformation was carried out as described by Svab *et al.* (1995). Multiple independent transgenic lines were generated with each of the binary constructs.

(c) Generation of T2 lines

T2 lines were generated from selected T1 lines by self-pollination. Tissue of young transgenic tobacco lines, containing the pGEL-1:GUS gene construct, were histochemically assayed to visualise GUS activity. Very intense levels of histochemical stain indicate high levels of expression of the GUS gene in tissues of young plants (Figure 7A, B).

(d) Quantitative analysis of pGEL-1

To quantify levels of expression of the GUS gene under control of pGEL-1 and compare it to levels obtained using the CaMV35S promoter, quantitative analysis was carried out on two independent transgenic T2 tobacco lines (3-4 and 7-3) containing the pGEL-1:GUS genetic construct and one transgenic T2 line (5-2) containing the 35S:GUS genetic construct. Assays were performed according to the method of Jefferson (1987) on different plant tissues including root, stem, petiole and first, second and third true leaves. The results indicated that constructs

containing pGEL-1 drive levels of expression two to five times higher than that obtained using the 35S promoter (see Figures 8, 9, 10 and 11).

(e) Deletion analysis

Several deletions of the pGEL-1 promoter regions were made and fused to the GUS gene ranging from 1.027 bp to 86 bp. Figure 11 shows the GUS activity measures performed in several plant organs at different developmental stages. It is observed that there is a general decline in activity in the shorter promoter constructs in immature and mature leaf tissue. Nevertheless. the decrease in activity is not so evident in other tissues.

EXAMPLE 6

Transformation procedures

The promoter is introduced into a range of plants generally from within a construct. Genetic material is introduced into the *Agrobacterium tumefaciens* strain AGL0 by adding 5 µg of plasmid DNA to 100 µl of competent AGL0 cells prepared by inoculating a 50 ml culture of MG/L (Garfinkel and Nester, 1980). These are cultured and grown for 16 hours with shaking at 28°C. The cells are then pelleted and resuspended in 0.5 ml of 85% v/v 100 mM $CaCl_2$/15% v/v glycerol. The DNA-*Agrobacterium* mixture is frozen by incubation in liquid N_2 for 2 minutes and then allowed to thaw by incubation at 37°C for 5 minutes. The DNA/bacterial mix is then placed on ice for a further 10 minutes. The cells are then mixed with 1 ml of LB (Sambrook *et al*, 1989) media and incubated with shaking for 16 hours at 28°C. Cells of *A. tumefaciens* carrying genetic material are selected on LB agar plates containing 10 µg/ml gentamycin or other suitable selection such as another antibiotic or a herbicide. The presence of genetic material is confirmed by Southern analysis of DNA isolated from the gentamycin-resistant transformants or any other selectable molecule such as another antibiotic or a herbicide.

Petunia transformations

(a) Plant material

Leaf tissue from mature plants is treated in 1.25% w/v sodium hypochlorite for 2 minutes and then rinsed three times in sterile water. The leaf tissue is then cut into 25 mm^2 squares and precultured on MS media (Murashige and Skoog, 1962) supplemented with 0.05 mg/l kinetin and 1.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) for 24 hours.

(b) Co-cultivation of *Agrobacterium* Tissue

Agrobacterium tumefaciens strain AGL0 containing genetic material is maintained at 4°C on MG/L agar plates with 100 mg/l gentamycin. A single colony is grown overnight in liquid medium containing 1% w/v Bacto-peptone, 0.5% w/v Bacto-yeast extract and 1% w/v NaCl. A final concentration of 5 x 10^8 cells/ml is prepared the next day by dilution in liquid MS medium containing B5 vitamins (Gamborg *et al,* 1968) and 3% w/v sucrose (BPM). The leaf discs were dipped for 2 minutes into BPM containing AGL0/genetic material. The leaf discs are then blotted dry and placed on co-cultivation media for 4 days. The co-cultivation medium consists of SH medium (Schenk and Hildebrandt, 1972) supplemented with 0.05 mg/l kinetin and 1.0 mg/l 2,4-D and included a feeder layer of tobacco cell suspension spread over the co-cultivation medium with a filter paper placed on top of the tobacco cell suspension.

(c) Recovery of transgenic plants

After co-cultivation, the leaf discs are transferred to a selection medium (MS medium supplemented with 3% w/v sucrose, α-benzylaminopurine (BAP) 2 mg/l, 0.5 mg/l α-naphthalene acetic acid (NAA), kanamycin 300 mg/l, 350 mg/l cefotaxime and 0.3% w/v Gelrite Gellan Gum (Schweizerhall)). Regenerating explants are transferred to fresh selection medium aft r 4 we ks. Adventitious shoots which survive the kanamycin selection are isolated and transferred to BPM containing 100 mg/l kanamycin and 200 mg/l cefotaxime for root induction. All

cultures are maintained under a 16 hour photoperiod (60 µmol. m^{-2} s^{-1} cool white fluorescent light) at 23± 2°C. When roots reach 2-3 cm in length the transgenic petunia plantlets are transferred to autoclaved Debco 51410/2 potting mix in 8 cm tubes. After 4 weeks, plants are replanted into 15 cm pots, using the same potting mix, and maintained at 23°C under a 14 hour photoperiod (300 µmol. m^{-2} s^{-1} mercury halide light).

EXAMPLE 7

Transformation of *Dianthus caryophyllus*

a. Plant material

Dianthus caryophyllus, (cv. Crowley Sim, Red Sim, Laguna) cuttings are used in this experiment. The outer leaves are removed and the cuttings are sterilized briefly in 70% v/v ethanol followed by 1.25% w/v sodium hypochlorite (with Tween 20) for 6 minutes and rinsed three times with sterile water. All the visible leaves and axillary buds are removed under the dissecting microscope before co-cultivation.

b. Co-cultivation of *Agrobacterium* and *Dianthus* tissue

Agrobacterium tumefaciens strain AGL0 containing a genetic construct encoding a cytochrome P450 monooxygenase and optionally an associated protein as herein described is maintained at 4°C on MG/L(Garfinkel and Nester, 1980) agar plates with 100 mg/l gentamycin. A single colony is grown overnight in liquid MG/L broth and diluted to 5 x 10^8 cells/ml the next day before inoculation. *Dianthus* tissue is co-cultivated with *Agrobacterium* on MS medium (Murashige and Skoog, 1962) supplemented with 3% w/v sucrose, 5 mg/l α-naphthalene acetic acid (NAA), 20 µM acetosyringone and 0.8% w/v Difco Bacto Agar (pH 5.7).

c. Recovery of transgenic *Dianthus* plants

Co-cultivated tissue is transferred to MS medium supplemented with 1 mg/l benzylaminopurine (BAP), 0.1 mg/l NAA, 150 mg/l kanamycin, 500 mg/l ticarcillin and 0.8% w/v Difco Bacto Agar (selection m dium). After three weeks, explants

are transferred to fresh selection medium and care is taken at this stage to remove axillary shoots from stem explants. After 6-8 weeks on selection medium, healthy adventitious shoots are transferred to hormone free MS medium containing 3% w/v sucrose, 150 mg/l kanamycin, 500 mg/l ticarcillin, 0.8% w/v Difco Bacto Agar. At this stage, GUS histochemical assay (Jefferson, 1987) and/or NPT II dot-blot assay (McDonnell *et al,* 1987) are used to identify transgenic shoots. Transgenic shoots are transferred to MS medium supplemented with 3% w/v sucrose, 500 mg/l ticarcillin and 0.4% w/v Gelrite Gellan Gum (Schweizerhall) for root induction. All cultures are maintained under a 16 hour photoperiod (120 μE cool white fluorescent light) at 23± 2°C. When plants are rooted and reached 4-6 cm tall they are acclimatised under mist. A mix containing a high ratio of perlite (75% or greater) soaked in hydroponic mix (Kandreck and Black, 1984) is used for acclimation, which typically lasts 4-5 weeks. Plants are acclimatised at 23°C under a 14 hour photoperiod (200 μE mercury halide light).

EXAMPLE 8

Transformation of *Rosa hybrida*

1. *Rosa hybrida* cv Royalty

Plant tissues of the rose cultivar Royalty are transformed according to the method disclosed in PCT/AU91/04412, having publication number WO92/00371.

2. *Rosa hybrida* cv Kardinal

a. Plant material

Kardinal shoots are used. Leaves are removed and the remaining shoots (5-6 cm) are sterilized in 1.25 % w/v sodium hypochlorite (with Tween 20) for 5 minutes followed by three rinses with sterile water. Isolated shoot tips are soaked in sterile water for 1 hour and precultured for 2 days on MS medium containing 3% w/v sucrose, 0.1 mg/l BAP, 0.1 mg/l kinetin, 0.2 mg/l Gibberellic acid, 0.5% w/v polyvinyl pyrrolidone and 0.25 % w/v Gelrite Gellan Gum, before co-cultivation.

b. Co-cultivation f *Agrobacterium* and *Rosa* shoot tissue

Agrobacterium tumefaciens strains ICMP 8317 (Janssen and Gardner, 1989) and AGL0, containing genetic constructs comprising pGEL-1 and optionally a structural gene operably linked thereto are maintained at 4°C on MG/L agar plates with 100 mg/l gentamycin. A single colony from each *Agrobacterium* strain is grown overnight in liquid MG/L broth. A final concentration of 5×10^8 cells/ml is prepared the next day by dilution in liquid MG/L. Before inoculation, the two *Agrobacterium* cultures are mixed in a ratio of 10:1. A longitudinal cut is made through the shoot tip and an aliquot of 2 μl of the mixed *Agrobacterium* cultures is placed as a drop on the shoot tip. The shoot tips are co-cultivated for 5 days on the same medium used for preculture.

Agrobacterium tumefaciens strain AGL0 is maintained at 4°C on MG/L agar plates with 100 mg/l kanamycin. A single colony from each *Agrobacterium* strain is grown overnight in liquid MG/L broth. A final concentration of 5×10^8 cells/ml is prepared the next day by dilution in liquid MG/L.

c. Recovery of transgenic *Rosa* plants

After co-cultivation, the shoot tips are transferred to selection medium. Shoot tips are transferred to fresh selection medium every 3-4 weeks. Galls observed on the shoot tips are excised when they reached 6-8 mm in diameter. Isolated galls are transferred to MS medium containing 3% w/v sucrose, 25 mg/l kanamycin, 250 mg/l cefotaxime and 0.25% w/v Gelrite Gellan Gum for shoot formation. Shoots regenerated from gall tissue are isolated and transferred to selection medium. GUS histochemical assay and callus assay are used to identify transgenic shoots. Transgenic shoots are transferred to MS medium containing 3% w/v sucrose, 200 mg/l cefotaxime and 0.25% w/v Gelrite Gellan Gum for root induction. All cultures are maintained under 16 hour photoperiod (60 μE cool white fluorescent light) at 23± 2°C. When the root system is well developed and the shoot reached 5-7 cm in length the transgenic rose plants are transferred to autoclaved Debco 514110/2 potting mix in 8 cm tubes. After 2-3 weeks plants are replanted into 15 cm pots

using the same potting mix and maintained at 23°C under a 14 hour photoperiod (300 μE mercury halide light). After 1-2 weeks potted plants are moved to glasshouse (Day/Night temperature : 25-28°C/14°C) and grown to flowering.

EXAMPLE 9

Transformation of *Chrysanthemum morifolium*

a. Plant material

Chrysanthemum morifolium (cv. Blue Ridge, Pennine Chorus) cuttings are obtained. Leaves are removed from the cuttings, which are then sterilized briefly in 70% v/v ethanol followed by 1.25% w/v sodium hypochlorite (with Tween 20) for 3 minutes and rinsed three times with sterile water. Internodal stem sections are used for co-cultivation.

b. Co-cultivation of *Agrobacterium* and *Chrysanthemum* tissue

Agrobacterium tumefaciens strain LBA4404 (Hoekema *et al*, 1983), containing a genetic construct of the present invention is grown on MG/L agar plates containing 50 mg/l rifampicin and 10 mg/l gentamycin. A single colony from the *Agrobacterium* is grown overnight in the same liquid medium. These liquid cultures are made 10% v/v with glycerol and 1 ml aliquots transferred to the freezer (-80°C). A 100-200μl aliquot of each frozen *Agrobacterium* is grown overnight in liquid MG/L containing 50 mg/l rifampicin and 10 mg/l gentamycin. A final concentration of 5 x 10^8 cells/ml is prepared the next day by dilution in liquid MS containing 3% w/v sucrose. Stem sections are co-cultivated with *Agrobacterium* in co-cultivation medium for 4 days.

c. Recovery of transgenic *Chrysanthemum* plants

After co-cultivation, the stem sections are transferred to selection medium. After 3-4 weeks, regenerating explants are transferred to fresh medium. Adventitious shoots which survive the kanamycin selection are isolated and transferred to MS medium containing kanamycin and cefotaxime for shoot elongation and root induction. All cultures are maintained under a 16 hour photoperiod (80 μE cool

white fluorescent light) at 23 ± 2°C. Leaf samples are collected from plants which rooted on kanamycin and Southern blot analysis is used to identify transgenic plants. When transgenic chrysanthemum plants reach 4-5 cm in length, they are transferred to autoclaved Debco 51410/2 potting mix in 8 cm tubes. After 2 weeks, plants are replanted into 15 cm pots using the same potting mix and maintained at 23°C under a 14 hour photoperiod (300 μE mercury halide light). After 2 weeks potted plants are moved to glasshouse (Day/Night temperature : 25-28°C/14°C) and grown to flowering.

EXAMPLE 10

Bombardment of plant tissue with genetic material comprising pGEL-1 operably linked to a gene of interest

The aim of these experiments is to introduce genetic constructs comprising pGEL-1 into plant tissue such as petals and then to screen for at least transient expression.

The gene bombardment protocol is initially optimised using the reporter vector pGEL-1:GUS. GUS expression is assayed using the method described by Jefferson *et al* (1992). Efficiency of the transformation is measured by the mean number of blue spots per petal bombardment. The parameters examined during these initial optimisation experiments are target distance, bombardment pressure and petal developmental stage.

Plasmid DNA is obtained from *E.coli* using a standard alkaline lysis procedure with and without additional procedures for purification of the resultant DNA (Sambrook *et al,* 1989). The DNA is prepared for bombardment by combining various amounts of tungsten particle solution with DNA. After vortexing, the particles are precipitated with $CaCl_2$ and spermidine. After removing a portion of the supernatant, the tungsten suspension was vortexed and an aliquot removed for bombardment.

In this experiment, white petunia flowers are used for bombardment. Petunia plants

having other colours may also be used. The device used for bombardment is the particle inflow gun developed by Finer *et al* (1992) which propels tungsten particles directly in a stream of helium towards the target. The petal is placed in a petri dish containing filterpaper moistened with appropriate medium. Each petal preparation is bombarded with one of:

a) vector containing pGEL-1 alone; or
b) vector containing pGEL-1 operably linked to GUS (or other gene of interest);
c) vector containing a GUS control (or other gene of interest).

In some cases, the vector containing the GUS control is bombarded simultaneously with either or both types of vectors containing pGEL-1.

The optimum petal distance and helium pressure found during these experiments is 12.5 cm shelf height and 1000 Kpa, respectively. Optimum DNA is about 2-5 ng DNA/petal. A negative control containing tungsten particles only is also included.

The success of the bombardment is analysed by the presence of blue spots after overnight incubation of the bombarded petal in the presence of GUS substrate.

EXAMPLE 11

Optimization of microprojectile bombardment of "Sunrise Solo" somatic embryos

A gene gun (based on the particle inflow gun; Finer *et al,* 1992) is used for bombardment. Tungsten particles (0.7 μm, Biorad) are used as microprojectiles; 16-20 mg tungsten is washed with ethanol and then washed three times with sterile double distilled water (ddH_2O) before suspension in 200 μl double distilled water. For preparation of microprojectiles, 100 μg/l tungsten suspension is mixed with 1 μg/l plasmid DNA, 2.5 mM $CaCl_2$ and 100 mM spermidine-free base. The plasmid

DNA used is, for example, p2.5GuNt (pGEL-1 promoter::GUS gene::Nos Terminator in a pBluescript backbone). However, any pGEL-1 construct may be used. For example, GUS may, of course, be replaced by a gene of interest. All solutions are kept on ice. The suspension is thoroughly mixed, then allowed to settle on ice for 5 minutes before 100 μl of the supernatant is removed and discarded. The remaining suspension is raked several times on the rack immediately before using 4 μl of the mixture for each bombardment. A protective buffle of nylon mesh (Franks and Birch, 1991) is placed over the tissue during bombardment. The tissues are bombarded using various pressures and distances. The bombarded embryos are then transferred onto a half-strength MS medium and incubated for 48 hours. After this period glucoronidase (GUS) activity is assayed histochemically by incubating the embryos in 8-bromo-4-chloro-3-indolyl glucoronide (X-gluc) solution overnight at 37°C (Jefferson, 1987). Transient expression is assayed 12 hours after incubation and measured as total blue foci count per shot area.

In experiment one, somatic embryos are placed on osmoticum medium (half strength MS salts and vitamins, 0.2 M mannitol and 0.5% w/v phytagel) for a total of six hours (three hours before and after bombardment). A protective buffle of nylon mesh (Franks and Birch, 1991) is placed over the tissue during bombardment. The tissues are bombarded using four different pressures (1000, 1500, 1800 and 2000 kPa). The distance of the target tissue from the filter unit containing the microprojectiles is 17.5 cm. The bombarded embryos are then transferred on a half-strength MS medium and incubated for 48 hours. After this period, GUS activity is assayed histochemically by incubating the embryos in 5-bromo-4-chloro-3-indolyl glucoronide (X-gluc) solution overnight at 37°C (Jefferson, 1987). Transient expression is assessed as total blue foci count per shot area.

In a second experiment, the somatic embryos produced from immature fruits are transferred onto a sterile filter paper (overlaided onto the medium) and are spread firmly over the surface of the filter paper with a sterile metal spatula in order to

squash the embryos (Gonsalves *et al*, 1997). The embryogenic cells are allowed to proliferate for another four to six weeks and are re-spread over the filter paper and, bombarded three days later as described above.

Three distances of the target somatic embryos from the filter containing the microprojectiles are tested. These distances are 17.5, 15.0 and 12.5 cm, and the pressure is 1000 kPa. The target tissues are bombarded following the procedure previously described.

EXAMPLE 12

Papaya transformation

Papaya tissue is transformed with genetic material using the following protocol. Growing temperatures are at 22-35°C.

1. Somatic Embryo Induction

Embryos are cut from immature (90 days old) papaya seeds and cultured on somatic embryo induction medium (SEIM) for 4-6 weeks or 3-4 months. The embryos are sub-cultured every 2 weeks on fresh SEIM. Seven to 12 embryos are then squashed using a metal spatula on 3MM filter paper, 3 days before shooting on SEIM.

2. Shooting

Embryos are placed, while still on the filter paper, onto osmoticum medium (OSM). Conveniently, this is done in the morning. The embryos are maintained on OSM for at least 8 hours before, during and after shooting.

a) Conditions for shooting are as follows:

Pressure: 1000 KPa

Distance target to filter: 12.5 cm
Pulse time: 50 msec

b) Tungsten Particles (0.7 µm): Particles are washed in ethanol 3 times, then 3 times in sterile double distilled H_2O (ddH_2O) and then resuspended to a final concentration of 100 µg/µl in ddH_2O.

c) DNA preparation:
The following components are added together:
50 µl Tungstein (100 µg/µl)
20 µl DNA (500 - 1000 ng total) [pPZP2.5GuNt or other suitable construct]
50 µl $CaCl_2$ (2.5 mM)
20 µl Spermidine (100 mM)

The latter two components are added in quick succession.

The mixture is allowed to sit for 5 min, for the tungsten to collect on the bottom and approximately 110 µl is removed from the top and discarded. This gives enough for 5 shots. Shots are made as quickly as possible because the DNA dissociates from tungsten.

d) Shooting:

Prior to shooting, the gun is swabbed together with the bench with alcohol. Tungsten-DNA is thoroughly resuspended and 4 µl is pipetted into the filter units Working aseptically, the baffle is placed onto the medium containing the tissue and slightly pressed into the agar. The filter is then screened into the gun. The gun chamber is evacuated until the vacuum gauge approximately reads -29mmHg and the fire button is pressed. The vacuum is immediately released and the tissue removed.

3. R c v ry

Embryos are placed, still on the filter paper onto recovery medium (RM) after shooting for 5 - 7 days.

4. Pre-selection

Embryos are removed from the filter paper and placed onto PSM for 1 month and sub-cultured every 2 weeks.

5. Full Selection

All embryos are placed onto a full selection medium (FSM) and sub-culture every 2-3 weeks. Tissue which is growing well is placed onto to FSM with 300 mg/l. kanamycin for two sub-cultures. Surviving tissue is placed onto EGM.

6. Regeneration

a) Embryo germination.

Embryos are placed onto embryo germination medium (EGM) with 150 mg/l kanamycin for 3-4 months (or longer until germinating clumps emerge). The embryos are sub-cultured every 2-4 weeks and maintained until green tissue emerges (1-2mm).

b) Single shoot growing.

Green tissue is placed onto full strength single shoot growing medium (SSGM) until a whole plant is obtained. Tissue is sub-cultured every month.

7. Micropropagati n

a) Shoot multiplication.

Stems are cut and leaves and roots removed and placed onto shoot multiplication medium (SMM) for 2 weeks up to one month.

b) Root Induction.

New emerging shoots are cut from the central shoot and placed onto root induction medium (RIM) for 3 days.

c) Shoots are placed onto full strength SSGM and sub-cultured every month until formation of a full grown plant.

d) The plant can be kept longer (up to one year) in culture using a minimal growth medium containing full strength SSGM plus 1% w/v fructose instead of glucose.

8. Potting out

The plants are planted out into Styrofoam seedling trays using steam sterilised soil. After 3 days, the seedling trays are drenched with a fungicide (eg. Dithane M45 or Alliette). These plants are placed in a humidifying chamber with the following acclimatisation conditions:

1st week	90-100% humidity
2nd week	70% humidity
3rd week	60% humidity
4th week	open door a bit

The plants are left in the chamber until the leaves become shiny. Plants are gently watered with distilled water when needed.

The following media are used:

1. Somatic embryo induction media (SEIM)

	1 litre
1/2 strength MS salts	2.17 g
MS Vitamins	1 ml (1000x stock)
2,4-D	10 ml (1 mg/ml stock)
Glutamine	20 ml (5 mg/ml stock)
Myo inositol	10 ml (1mg/ml stock)
thiamine HCl	10 ml (1 mg/ml stock)
Sucrose	30 g
Agar	8 g
or Phytagel	5 g
pH 6.5 - 7	

MS Vitamins(1000x):	100 ml
Stored frozen	
Thiamine-HCl	10 mg
Pyridoxine-HCl	50 mg
Nicotinic acid	50 mg
Glycine	200 mg
Myo-inositol	10 g

2. Osmoticum media (OSM)

	1 litre
1/2 strength MS salts	2.17 g
1/2 MS Vitamins	500 µl (1000x stock)
Mannitol	36.4 g

Agar	8 g
or Phytagel	5 g
pH 6.5 - 7	

3. Recovery media (RM)

	1 litre
1/2 strength MS salts	2.17 g
1/2 MS Vitamins	500 µl (1000x stock)
Sucrose	30 g
Agar	8 g
or Phytagel	5 g
pH 6.5 - 7	

4. Pre-selection (PSM)

SEIM with 75 mg/l kanamycin (750 µl of a 100 mg/ml stock in 1 litre)

5. Full selection media (FSM)

SEIM with 150 mg/l kanamycin (1500 µl of a 100 mg/ml stock in 1 litre) or 300 mg/ml kanamycin (3000 µl of a 100 mg/ml stock in 1 litre)

7. Embryo germination media (EGM)

	1 litre
1/2 Strength MS salts	2.17 g
1/2 MS Vitamins	500 µl (1000x stock)
Kinetin	0.25 µM (2.5 ml of a 100 µM stock)
IAA	4.5 µM (45 ml of a 100 µM stock)
GA3	0.8µM (8 ml of a 100 µM stock, filter sterilised, added after autoclaving)

Sucrose	30 g
Agar	8 g
or Phytagel	5 g
pH 6.5 - 7	

After autoclaving add 1500 µl of a 100 mg/ml stock in 1 litre

8. Single shoot growing media (SSGM)

Full strength SSGM	1 litre
De Fossard's Minerals	80 ml (1X)
De Fossard's Vitamins	50 ml (2X)
Sucrose	30 g
Agar	8 g
or Phytagel	5 g
pH 6.5 - 7	

9. Shoot multiplication media (SMM)

	1 litre
De Fossard's Minerals	80 ml (1X)
De Fossard's Vitamins	50 ml (2X)
Sucrose	30 g
0.25µM BAP	2.5 ml of a 100 µM stock
0.25µM NAA	250 µl of a 1000 µM stock
Agar	8 g
or Phytagel	5 g
pH 6.5 - 7	

10. Ro t induction media (RIM)

1 litre

De Fossard's Minerals	40 ml (1X)
De Fossard's Vitamins*	50 ml (1X)
Sucrose	30 g
10 µM IBA	10 ml of a 1000 µM stock
Agar	8 g
or Phytagel	5 g
pH 6.5 - 7	

*De Fossards vitamins with no riboflavin

De Fossards Minerals (1X)	2.4 litre
NH_4NO_3	300 ml
KNO_3	600 ml
$NaH_2PO_4.H_2O$	300 ml
$CaCl_2$	300 ml
(Ferric sodium salt) FeNaEDTA	300 ml
$MgSO_4.7H_2O$	300 ml
Micronutrients	300 ml

Vitamins #6 (2X)	2 litre
Inositol	4.32 g
Nicotinic acids	196 mg
Pyridoxine HCl (100 mg/ml)	496 µl
Thiamine HCl	539 mg
Biotin (50 mg/ml)	200 µl
Folic acid (50 mg/ml)	712 µl
Ca-Pantothenate (50 mg/ml)	1910 µl
Riboflavin	150.8 mg
Ascorbic acid (100 mg/ml)	704 µl
Choline chloride (100 mg/ml)	560 µl
Glycine (100 mg/ml)	1504 µl

L-Cysteine HCl	756 mg

Stock Solutions	g/litre
NH_4NO_3	160.1 (2 M)
KNO_3	101.11 (1 M)
$NaH2PO4.H_2O$	31.202 (0.23 M)
$CaCl_2$	59.46 (0.54 M)
(Ferric sodium salt)	
FeNaEDTA	3.67 (0.01 M)
MgSO4.7H2O	73.95 (0.3 M)

Micronutrients	1 litre
H_3Bo_3	0.9276 (0.015 M)
$MnSO_4.4H_2O$	2.2306 (0.01 M)
$ZnSO_4.47H_2O$	1.1502 (4×10^{-3} M)
$CuSO_4.5H_2O$	0.0374 (1.5×10^{-4} M)
Ammonium Molybdate	
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.1236 (1×10^{-4} M)
$CoCl_2.4H_2O$	0.0238 (1×10^{-4} M)
KCl	0.0830 (5×10^{-4} M)

Vitamin Stocks	
Pyridoxine HCl (100 mg/ml)	1.5 g/15 ml in H_2O/ETOH
Biotin (50 mg/ml)	750 mg/15 ml dil HCl
Folic acid (50 mg/ml)	750 mg/15 ml dil NaOH
Ca-Pantothenate (50 mg/ml)	750 ml/15 ml H_2O
Ascorbic acid (100 mg/ml)	1.5 g/15 ml H_2O
Choline chloride (100 mg/ml)	1.5 g/15 ml H_2O
Glycine (100 mg/ml)	1.5 g/15 ml H_2O

De Fossard media (full strength) contains (in 1 litre)

NH_4NO_3	10 ml
KNO_3	20 ml
$NaH_2PO_4.H_2O$	10 ml
CaCl2	10 ml
FeNaEDTA	10 ml
$MgSO_4.7H_2O$	10 ml
Miconutrients	10 ml
Vitamins #6 (1X)	100 ml (50 ml of 2X)

EXAMPLE 13

Transformation of cotton, *Brassica* and maize

Genetic constructs comprising pGEL-1 or a functional derivative or homologue thereof operably linked to a gene of interest, such as, for example, a reporter gene, are introduced into cotton, *Brassica* (e.g. canola) and maize. Cotton is transformed using *Agrobacterium* using the method described in US Patent No. 5, 004, 863. *Brassica* sp are transferred using *Agrobacterium* using the method described in US Patent No. 5, 188, 958. Maize is transformed *via* immature embryos using the method described in US Patent No. 5, 641, 664. These plants may also be transformed using electroporation, biolistic procedures and polyethylene glycol amongst other methods.

EXAMPLE 14

Transformation of wheat, barley and rice

Wheat transformation was by the method of Karunaratne *et al* (1996) with slight modifications.

Target tissue

Young caryopsis are dissected from spikes of *Triticum aestivum* L. cv. Hartog,

approximately 12 to 14 days post anthesis and surface sterilised with 10% w/v Dairy-Chlor (100 g/l available chlorine). Immature embryos are isolated and cultured in dark on MS medium (Murashige and Skoog, 1962) supplemented with 2, 4-dichlorophenoxyacetic acid (10 μM). After 7 days of culture, the immature embryos are subjected to particle bombardment.

Microprojectile bombardment

The genetic construct to be introduced into plant cells is precipitated onto tungsten particles (1.2 μm) as descried by Finer and McMullen (1990) with the following modifications. An aliquot of 25 μl of a 500 mg/ml suspension of tungsten particles (1.2 μm) in distilled water is taken in an eppendorf tube followed by stepwise addition of the following: 5 μl of plasmid DNA (5 μg), 25 μl of calcium chloride (2.5 M), 10 μl of spermidine (0.1 M). The contents in the tube is mixed by finger vortexing and kept on ice. After 5 min, 30 μl of the supernatant is discarded and 300 μl of ethanol (90%) is added and kept on ice after mixing the contents. After 1 min, the tube is centrifuged and all the supernatant discarded. The ethanol wash is repeated once and the DNA-coated tungsten is finally suspended in 30 μl of ethanol (90%). The DNA-coated tungsten particles (2 μl) are delivered to the target tissue using a particle inflow gun (Finer *et al,* 1992). The target tissue is placed on a shelf 14 cm from the screen of the filter holder, which carried a suspension of plasmid-DNA coated tungsten particles. After bombardment, the tissue is transferred to the original medium and cultured in the dark for 2 months with fortnightly subculture.

Plant reg neration and selection

Embryogenesis leading to plant regeneration is stimulated by transferring clumps of embryogenic callus to MS medium devoid of hormones and containing Phosphinotricin (PPT) at a concentration of 5 mg/l. After two weeks, PPT-resistant plants and callus is transferred to fresh medium and subcultured weekly. PPT-resistant plants which are 4-5 cm are transferred to soil and kept under water mist for two weeks. Plants are then transferred to larger pots and kept in the glasshouse under day and night temperature of 22°C and 19°C, respectively.

Rice is transformed by the method of Abedinia *et al* (1997). Barley is transformed according to the method of Tingay *et al* (1997).

Hormone stocks			
Hormone	**Molecular Weight (g)**	**mg/l Stock**	**Concentration of stock**
BAP	225.2	22.6	100 µM
NAA	186.2	186.2	1000 µM
IAA	175.2	17.5	100 µM
GA3	346.4	34.6	100 µM
Kinetin	215.2	21.5	100 µM
IBA	203.23	203.2	1000 µM

EXAMPLE 15

Southern analysis of Transgenic T2 tobacco lines

Genomic DNA (10 µg) was digested with *Eco*RI or *Bam*HI restriction enzymes; separated in an electrophoresis gel and transferred to a Hybond™ (Amersham) Nylon membrane. The membrane was prehybridized and hybridized at high stringency following standard procedures (Sambrook *et al,* 1989). A DNA fragment containing the full GUS gene and Nos terminator was labelled with ^{32}P and used as a probe. After washing at high stringency the following results were observed:

a) The *Eco*RI lanes of lines 3-4, 7-3 and 10-3 show a single fragment of the expected 4.5kb size indicating the intactness of the GEL-1:GUS:NosT construct in each of these lines.

b) The *Bam*HI lanes of lines 3-4, 7-3 and 10-3 show single fragments of different sizes (one fragment per line) indicating the existence of a single copy of GUS:NosT construct in each of these lines.

c) The *Bam*HI lane of line 5-2 shows two bands indicating that this line contains two copies of the CaMV 35S:GUS:NosT portion of the construct.

These results are shown in Figure 13

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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CLAIMS:

1. An isolated nucleic acid molecule comprising a sequence of nucleotides or complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof, wherein, in its native form, the promoter is inducible in response to physical stimulation.

2. An isolated nucleic acid molecule according to claim 1 wherein, in its native form, the promoter directs expression of a gene associated with ethylene production.

3. An isolated nucleic acid molecule according to claim 2 wherein the promoter, in its native form, directs expression of a gene encoding 1-aminocyclopropane-1-carboxylic acid (ACC) synthase.

4. An isolated nucleic acid molecule according to any one of claims 1 to 3 wherein the promoter is selected from the group consisting of:

(i) a promoter which directs expression of a nucleotide sequence as substantially set forth in <400>1;

(ii) a promoter which directs expression of a nucleotide sequence which hybridizes under low stringency conditions to <400>1;

(iii) a promoter which directs expression of a nucleotide sequence having at least about 50% similarity to <400>1;

(iv) a promoter which directs expression of a nucleotide sequence which encodes an amino acid sequence substantially as set forth in <400>2;

(v) a promoter which directs expression of a nucleotide sequence which

ncodes an amino acid sequence which has at least about 60% similarity to <400>2.

5. An isolated nucleic acid molecule according to any one of claims 1 to 4 comprising a nucleotide sequence substantially as set forth in <400>3 or a nucleotide sequence having at least 25% similarity thereto or a nucleotide sequence capable of hybridising to <400>3 under low stringency conditions.

6. An isolated nucleic acid molecule comprising a sequence of nucleotides or complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof, wherein, in its native form, the promoter is inducible in response to physical stimulation and wherein the promoter is selected from the list consisting of:

(i) a promoter which, in its native form, directs expression of a nucleotide sequence substantially as set forth in <400>1;

(ii) a promoter which, in its native form, directs expression of a nucleotide sequence which hybridizes under low stringency conditions to <400>1;

(iii) a promoter which, in its native form, directs expression of a nucleotide sequence having at least about 50% similarity to <400>1;

(iv) a promoter which, in its native form, directs expression of a nucleotide sequence which encodes an amino acid sequence substantially as set forth in <400>2;

(v) a promoter which, in its native form, directs expression of a nucleotide sequence which encodes an amino acid sequence which has at least about 60% similarity to <400>2;

(vi) a promoter comprising a nucleotide sequence substantially as set forth in <400>3;

(vii) a promoter comprising a nucleotide sequence capable of hybridizing to <400>3 under low stringency conditions; and

(viii) a promoter comprising a nucleotide sequence having at least about 25% similarity to <400>3.

7. A nucleic acid molecule defining a promoter or a homologue or derivative thereof said nucleic acid molecule obtainable by the method of isolating genomic DNA from plant cells, rendering the genomic DNA or portion thereof single stranded and then identifying a region on genomic DNA which hybridizes to a primer corresponding to all or part of <400>1 or a complementary form thereof and the cloning DNA upstream of the region of primer hybridization.

8. A nucleic acid according to claim 7 alternatively comprising amplifying regions of single stranded genomic DNA with a primer corresponding to all or part of <400>1 or a complementary form thereof and then cloning DNA upstream of the amplified region.

9. An isolated promoter obtainable by the method of:

(i) amplifying a region of single stranded plant genomic DNA with the primers <400> 4 and <400>5;

(ii) optionally amplifying the amplified DNA of (i) above with primers selected from <400> 6 and <400>7 or <400> 8 and <400>9;

(iii) running amplified DNA on a gel and excising the product of amplification; and

(iv) subcloning product and identifying the promoter.

10. A nucleic acid according to claim 7 or 8 or a promoter according to claim 9 comprising a nucleotide sequence substantially as set forth in <400>3 or a nucleotide sequence having at least 25% similarity thereto or a nucleotide sequence capable of hybridising to SEQ ID NO:3 under low stringency conditions.

11. A genetic construct comprising a nucleic acid molecule defining a promoter according to any one of claims 1 to 10.

12. A genetic construct according to claim 11 further comprising a structural or regulatory gene operably linked to said promoter.

13. A method of altering a characteristic of a plant said method comprising introducing a genetic construct according to claim 12 into a cell or group of cells of a plant and wherein said structural or regulatory gene facilitates the altering of said plant characteristic, regenerating a plant or plantlet from said cell or group of cells carrying said genetic construct and growing or subjecting said plant or plantlet to conditions sufficient to induce the promoter in said genetic construct.

14. A method according to claim 13 wherein the altered plant characteristic comprises resistance to a plant pathogen, altered nutritional characteristics, expression of a plantabody, an altered biochemical pathway, altered fertility and/or altered flower colour.

15. A modular promoter, said modular promoter comprising at least one portion which is derived from a promoter which, in its native form, directs expression of a gene associated with ethylene biosynthesis and is inducible by physical stimulation.

16. A modular promoter according to claim 15 wherein the native promoter directs expression of a gene encoding 1-aminocyclopropane-1-carboxylic acid

(ACC) synthase.

17. A modular promoter according to any one of claims 15 to 16 wherein the native promoter is selected from the group consisting of:

(i) a promoter which directs expression of a nucleotide sequence as substantially set forth in <400>1;

(ii) a promoter which directs expression of a nucleotide sequence which hybridizes under low stringency conditions to <400>1;

(iii) a promoter which directs expression of a nucleotide sequence having at least about 50% similarity to <400>1;

(iv) a promoter which directs expression of a nucleotide sequence which encodes an amino acid sequence substantially as set forth in <400>2;

(v) a promoter which directs expression of a nucleotide sequence which encodes an amino acid sequence which has at least about 50% similarity to <400>2.

18. A modular promoter according to any one of claims 1 to 4 comprising a nucleotide sequence substantially as set forth in <400>3 or a nucleotide sequence having at least 25% similarity thereto or a nucleotide sequence capable of hybridising to <400>3 under low stringency conditions.

19. A transgenic plant comprising a nucleic acid molecule according to any one of claims 1 to 9.

20. A vegetative or reproductive portion of a transgenic plant according to claim 19.

21. A cut or severed flower from a transgenic plant according to claim 19.

AIM-1 OLIGONUCLEOTIDES

-Oligonucleotide primers used during Long Distance Inverse PCR
-Oligo's bind to regions of AIM-1 (Mungbean ACC Synthase)

NSE-1

5` -GCGGAT↓CCATCTTGGACAACAAGGGAGTT- 3`
29'omer
Tm = 68

NSE-2

5` -TAGGATC↓CAGAAAGACACTGAGAACCGTGG- 3`
30'omer
Tm = 70

NSE-3

5` -ACGGATCC↓GGTGTATGTGGTTAGAGTGTG- 3`
29'omer
Tm = 62

NSE-4

5` -CAGGATC↓CAGACATAGAGTGTGACCGCAA- 3`
29'omer
Tm = 66

NSE-5

5`-ATCGATCATATGAGCTCTAGACCCGGGCTGCAGGATCC↓GGTGTATGTGGTTAGAGTGTG- 3`
59'omer
Tm = 62
note: NSE-5 is identical to NSE-3 except different restriction enzyme sites have been incorporated (ie. 5`-Cla I, Nde I, Sac I, Xba I, Sma I, Pst I & Bam H1-3`)

NSE-6

5`-CCGCGGAGATCTATCGATCTCGAGAATTCAAGCTT↓CAGACATAGAGTGTGACCGCAA-3`
57'omer
Tm = 66
note: NSE-6 is identical to NSE-4 except different restriction enzyme sites have been incorporated (ie. 5`-Sac II, Bgl II, Cla I, Xho I, Eco R1, & Hind III-3`)

FIGURE 1

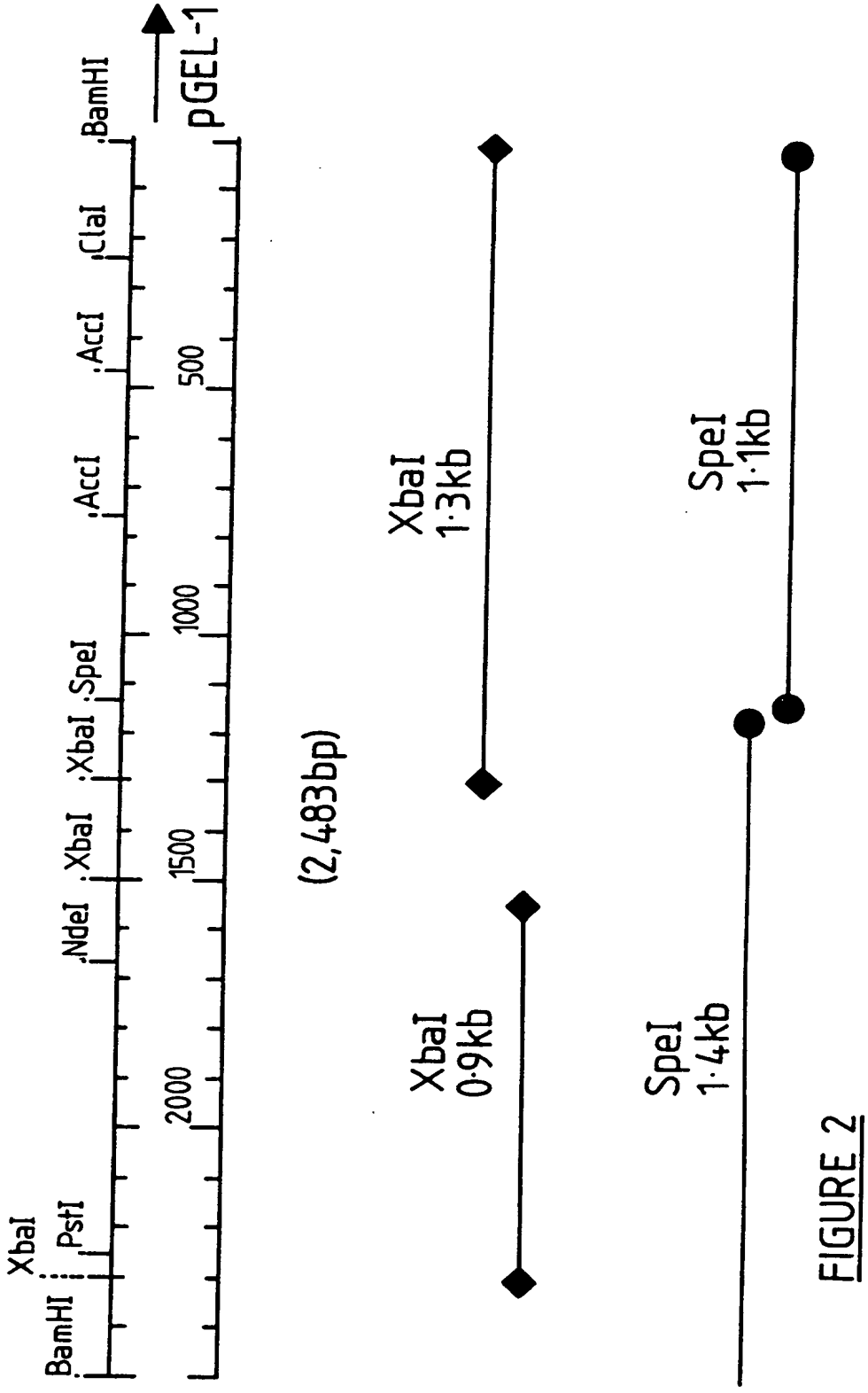

pGEL-1
BamHI
ClaI
AccI
AccI
SpeI
XbaI
XbaI
NdeI
PstI
XbaI
BamHI
500
1000
1500
2000
(2,483bp)
XbaI
1·3kb
XbaI
0·9kb
SpeI
1·1kb
SpeI
1·4kb

FIGURE 2

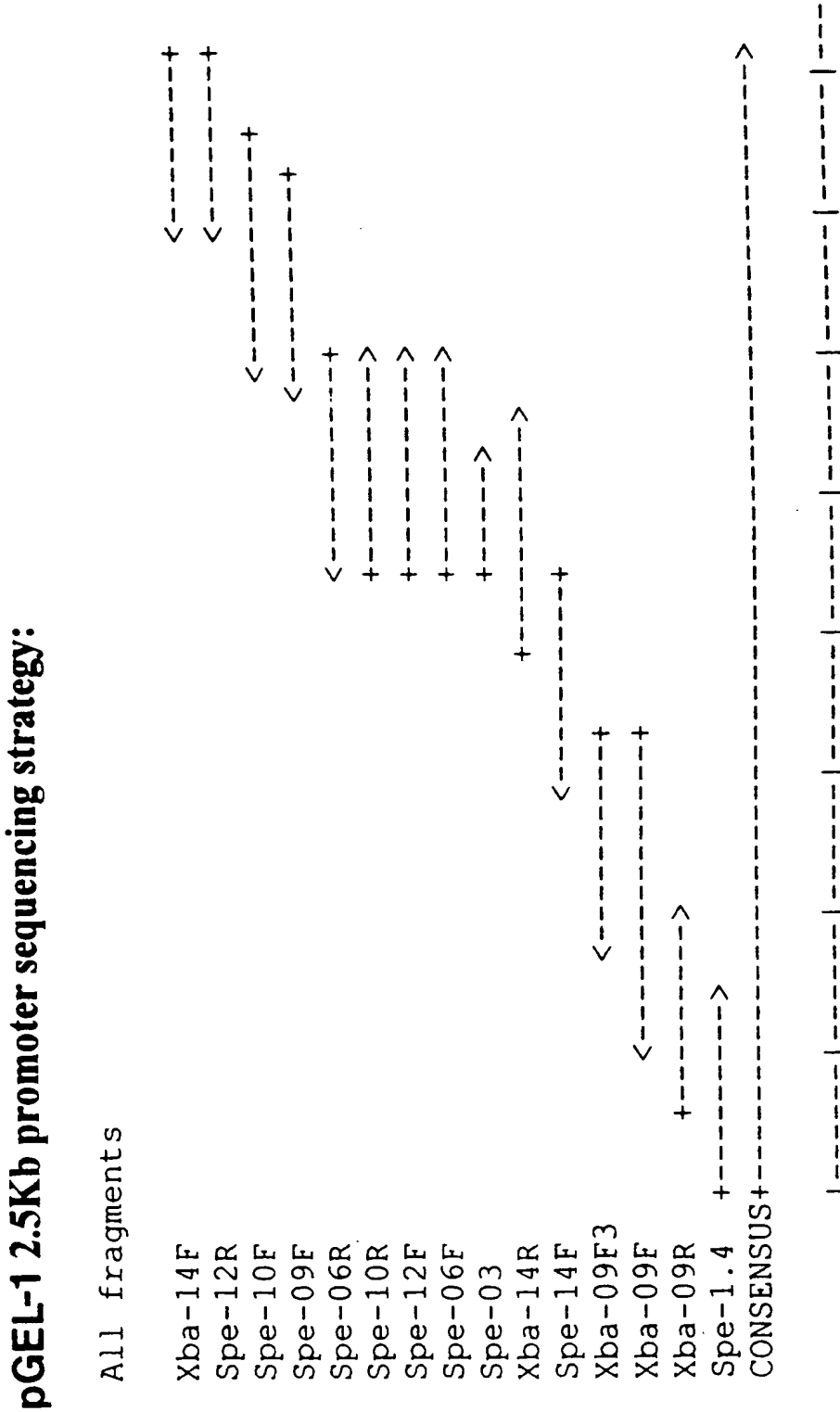

pGEL-1 2.5Kb promoter sequencing strategy:
All fragments
Xba-14F
Spe-12R
Spe-10F
Spe-09F
Spe-06R
Spe-10R
Spe-12F
Spe-06F
Spe-03
Xba-14R
Spe-14F
Xba-09F3
Xba-09F
Xba-09R
Spe-1.4
CONSENSUS
0
300
600
900
1200
1500
1800
2100
2400

FIGURE 3

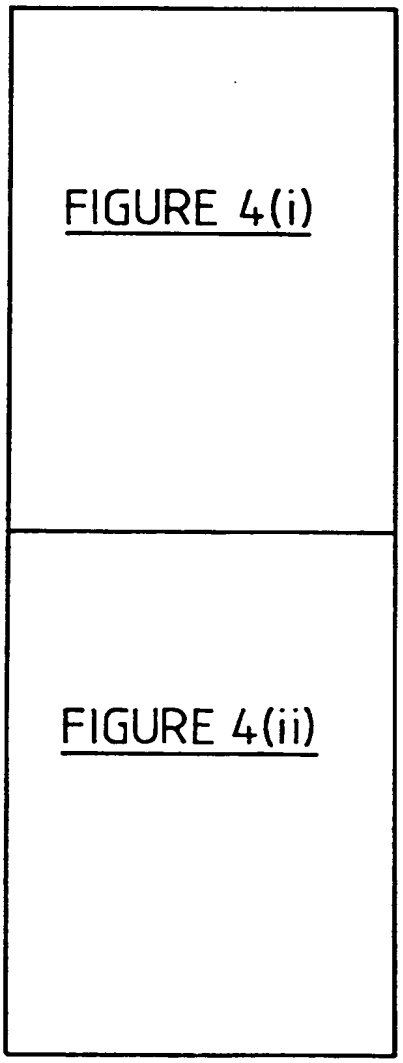


FIGURE 4

pGEL-1 2.5Kb Promoter Fragment

Length: 2470

```
   1  TTACAGATAC ACAGAATCAG ACGACACATC TACTTTAATA ACAGAAAAAT
  51  AATAAGTGTC GGAGATTATG GTACGACAAG ATGAAATGTT TTTATATGGT
 101  TGAGATTATT TTGGTCTGTT GTTGGAAGTT TCACGAATCA TGATTTTGAT
 151  TTTACGTATT AAAAAATGAA AAGTTGAATC ATGCATTTTA TCTAGAAGCT
 201  GGGAACTGAA CCAAAAAAAT AGCCAGTTGA ACAACTGCAG TATTTGTAGG
 251  CGTATTCATT TCTCCTTTCC TACAATAATC CTTGGTTGCT CTTTATCGGA
 301  AAAAAACCAA AAGCAATAGC TACTCTGTAA GGTCCTCGAT TGCCGACAAG
 351  AACATCACAT GCGTGCTGTC GAAGAACACA TAATTTTGAG GTTGAAGCTC
 401  ACGTGCGAGT TTTGCATATT TTTAGGTTAT GTGTACACGT ATGGAGTGAG
 451  TTCCGCGTAT ATAGTGTAGG TAGTTGAGTG GCTGAGTAGC GAGTGAATCA
 501  GGTAACACTA TCTTTTCAAG CCACCTAATT AAGGGATTTA ATGTTCATGC
 551  AACTGTTCTT CGCTAACTAA GGCCCCACTT ACCTTTATAA TATTCTCTCT
 601  AACTCCGGGC TTTTGGTAAG TACAACTTTT CTACTCTTAT TTAATGGAGG
 651  GATTATTTTT TCCATATACC AATTAATTTA TTTTTTAATT TATGCATTTT
 701  GATCTTATAT TAAAACAATT ATGGTATGGA TTAAGTCGTA TATCGGTGAC
 751  AATTGAAGTT TTCCTCAAGT TTAGCCATTT TTATGAAATT AAACTTAATC
 801  ACTACTATTA GGTAAATTCA TATGTATCAT TAACAATTTC AATGTGAGTT
 851  CAATTTTACC CAAGATTTGA AAGTTGTTGT CAACTTCTGT TAACTAAAGT
 901  TGTATTATAA GGTTGACGAC TTTAACCTAA ATCTATTTTG AATTGAAGGG
 951  GTTGATGACT TCAGCTTTAA AATAATTCAA CTAAAGTTCT AGACTACATT
1001  GGAGATTTTA GTGTTCATAA AATTTTAGAA AAAGGCTGAG TTAAAGTTAT
1051  GAAAAAGATT GGTGACTATT CAATTAATTA GTTGTGAATT GATGACAAAT
1101  ATTTCATGAG CATAACCAAT CAGAGAAATA CCACCTCGAC CGACTACAAC
1151  AATCTCAATG TTAATTAATG AAGCATTGTA GTATAAGGAG TCTAGAATAA
1201  ATTTCTTAAA TATTAGAGGA AAACTATTTT TAAAAAATTA CAAGAAAAGT
1251  TTGATCTATA ACCTCTTTAA ACTTTAAATT ATCTAACAAT TTTCTTATGA
```

FIGURE 4(i)

1301 CTCACATTGT GTTGATAGGG TGATTTTGTC AAAATATATG TCTATTTTAT

1351 ACTAGTATGA TTTGTCTGCG AATTATATAT AGTATTAACT TGGAGAAATG

1401 ATTGCCTAAT AAGTTATAAA AAAGGAGAAA ATATTTATTC ATAAAAAAAA

1451 TACACTTAAA TAAGTAACAA TAATAAAAAA CATTATATAA GAGATTAAGA

1501 TAATTTAATA AGTATTGAAT GTAGAATAAT TTTTATTTAT AAATTTGAAC

1551 TAAAATATTC AAATAATATT CAAAGTAAAT AATAGATATA ATTCATCATT

1601 CAATACGAGT AATTCAATCT ATTATAATCC ATATATTAGA TAAATATACA

1651 AATATTTGTT AAATTTTACA TTATTATATT ACTAAATATA TATTAATTTC

1701 CTTTGAATAT CTTTTATACA AGTAGGTAGA CTAGAAGAAT TATCTTATCT

1751 CCCGTATATT TGTAGATGTT AAATGTAACG GGCTTAGACT GATGTTTTTG

1801 TATTATATTA TTTATAAATC CATTAGAGAT TTAAGTTAAT GTCTCTCTTT

1851 GATTTTAAAC ATGGTCTAAA AATTAGGTTT AATCATTGCG TCCTCAATGA

1901 ACCCATGCTA TATGTTTTAA AGTTTTTTGT TTTTTGACAA TGTTTTTTAT

1951 TTCTGAGATT GCTCTTAGGA TTGAAATTAT GTTTGATACT AGAAAACGAA

2001 GAAGTAGAGA GTAGTGTATA CACGTGTAAA AAATAATAGT TGTGGGAACT

2051 TAAGTTGGAT TTGAATACTA GGACGAGGCT GGAAGGGTTT CCACTAAGTT

2101 GACAAAAATT ATTACAAGTG GCAACTAGCT AGGTCTCACA AAGTATTACT

2151 AATTAATAGT GGGTCTGTCT GCATACCAAC TCTTGCCTAA TTTTCAAACA

2201 CCGCATTCTC TCTTCTTCTC TCCTTCTTCC TCTGGAAACT TCATCGATGT

2251 GGACTTCTGT CTCTCAAAAG TCAAGCTCAA TTTATCCAAT GCATTATAAA

2301 TACACACTCT CCCTCCCTTC TATTCTTCAT TGCATCACAT TTCCTCTATA

2351 AATTACTCAC ACCTTATTCC TAACTTCATT TCAACATCCT CTCTCCCACT

2401 TACTTCGATT TCATCAATTC CAATAAACTC AACACACTTT TTTACACTCC

2451 ACACTCTAAC CACATACACC

FIGURE 4(ii)

7/28

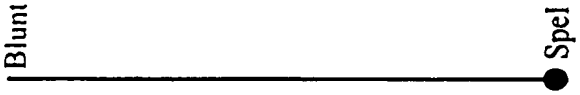
Reconstruction of 2.5 kb **pGEL-1** promoter

(a)

1. Cut HindIII and blunt end pGS1.4

pGS1.4

2. Cut SpeI



(b)

1. Cut SalI and blunt end pGS1.1

pGS1.1

2. Digest with SpeI

pGS1.1

(c) Ligate (a) into (b)

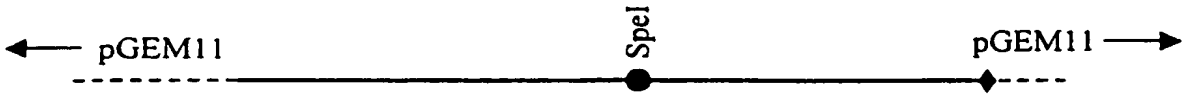


FIGURE 5

pGS2.5

FIGURE 6A(i)

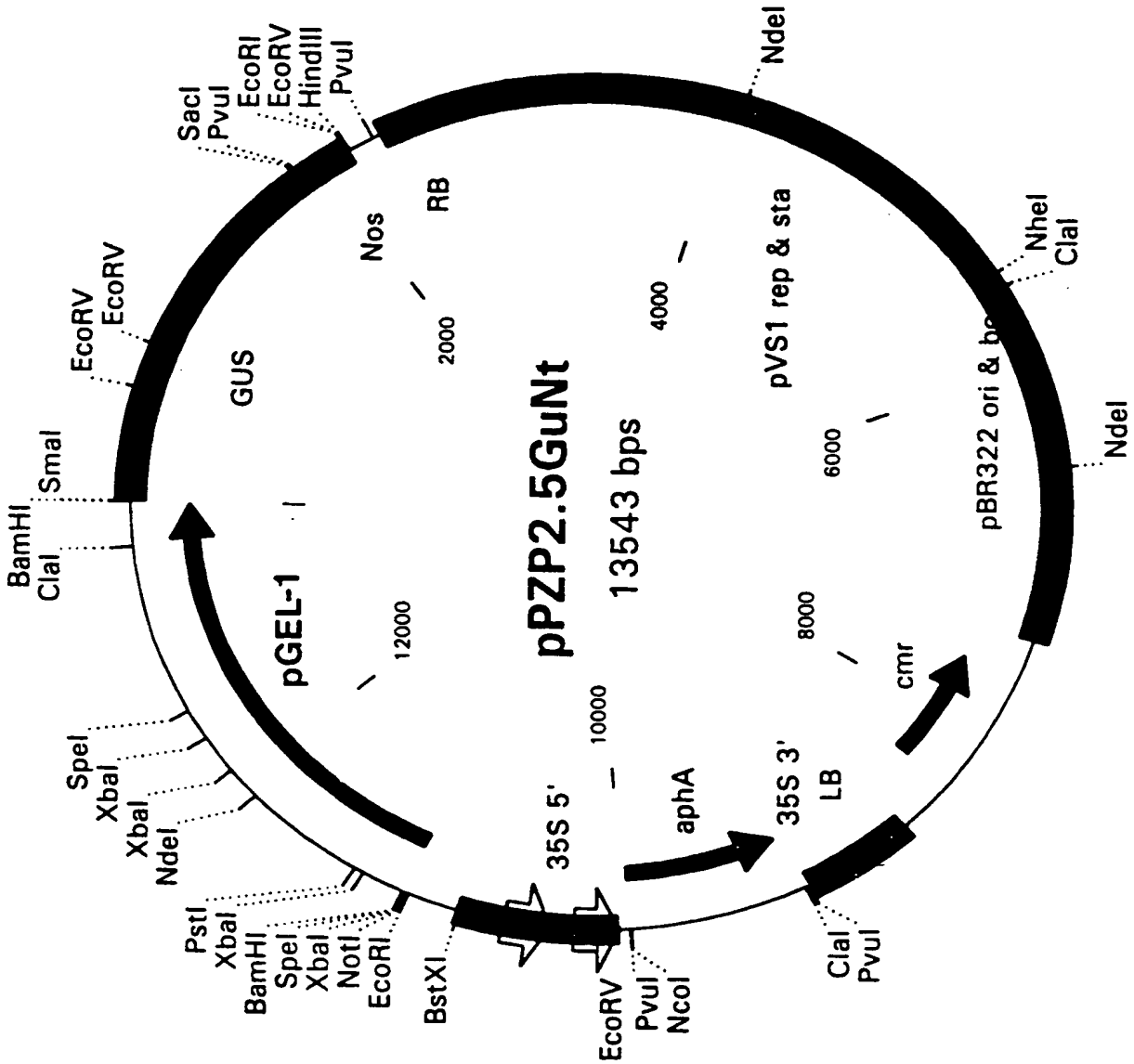

pPZP2.5GuNt
13543 bps
pGEL-1
GUS
Nos
RB
pVS1 rep & sta
pBR322 ori & bo
aphA
35S 5'
35S 3'
LB
cmr
2000
4000
6000
8000
10000
12000
SacI
PvuI
EcoRI
EcoRV
HindIII
PvuI
NdeI
NheI
ClaI
NdeI
ClaI
PvuI
EcoRV
PvuI
NcoI
BstXI
EcoRI
NotI
XbaI
SpeI
BamHI
XbaI
PstI
NdeI
XbaI
SpeI
EcoRV
EcoRV
SmaI
BamHI
ClaI

FIGURE 6A(ii)

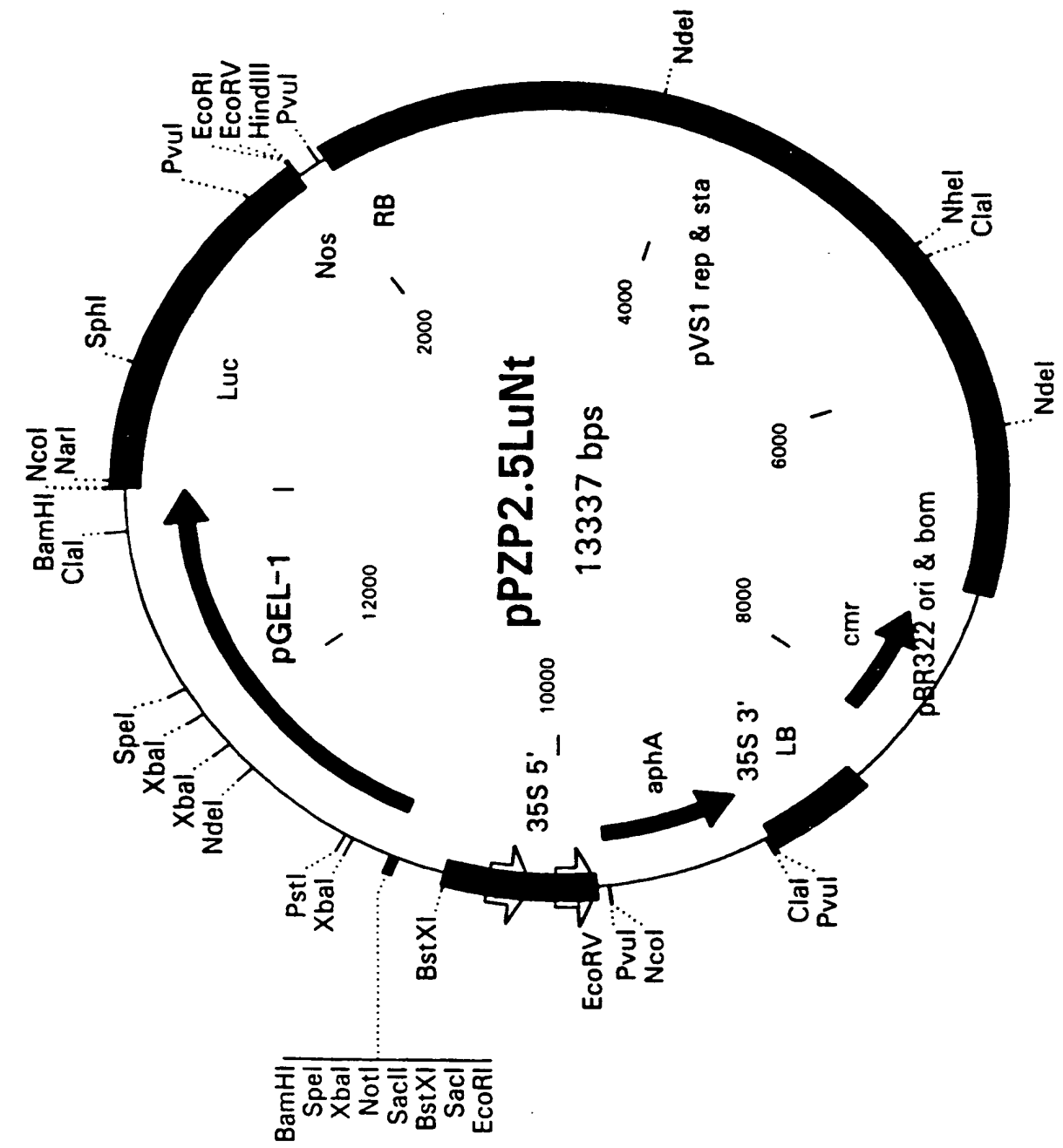

pPZP2.5LuNt
13337 bps
pGEL-1
Luc
Nos
RB
pVS1 rep & sta
pBR322 ori & bom
cmr
aphA
35S 5'
35S 3'
LB
2000
4000
6000
8000
10000
12000
NcoI
NarI
BamHI
ClaI
SphI
PvuI
EcoRI
EcoRV
HindIII
PvuI
NdeI
NheI
ClaI
NdeI
SpeI
XbaI
XbaI
NdeI
PstI
XbaI
BstXI
EcoRV
PvuI
NcoI
ClaI
PvuI
BamHI
SpeI
XbaI
NotI
SacII
BstXI
SacI
EcoRI

FIGURE 6A(iii)

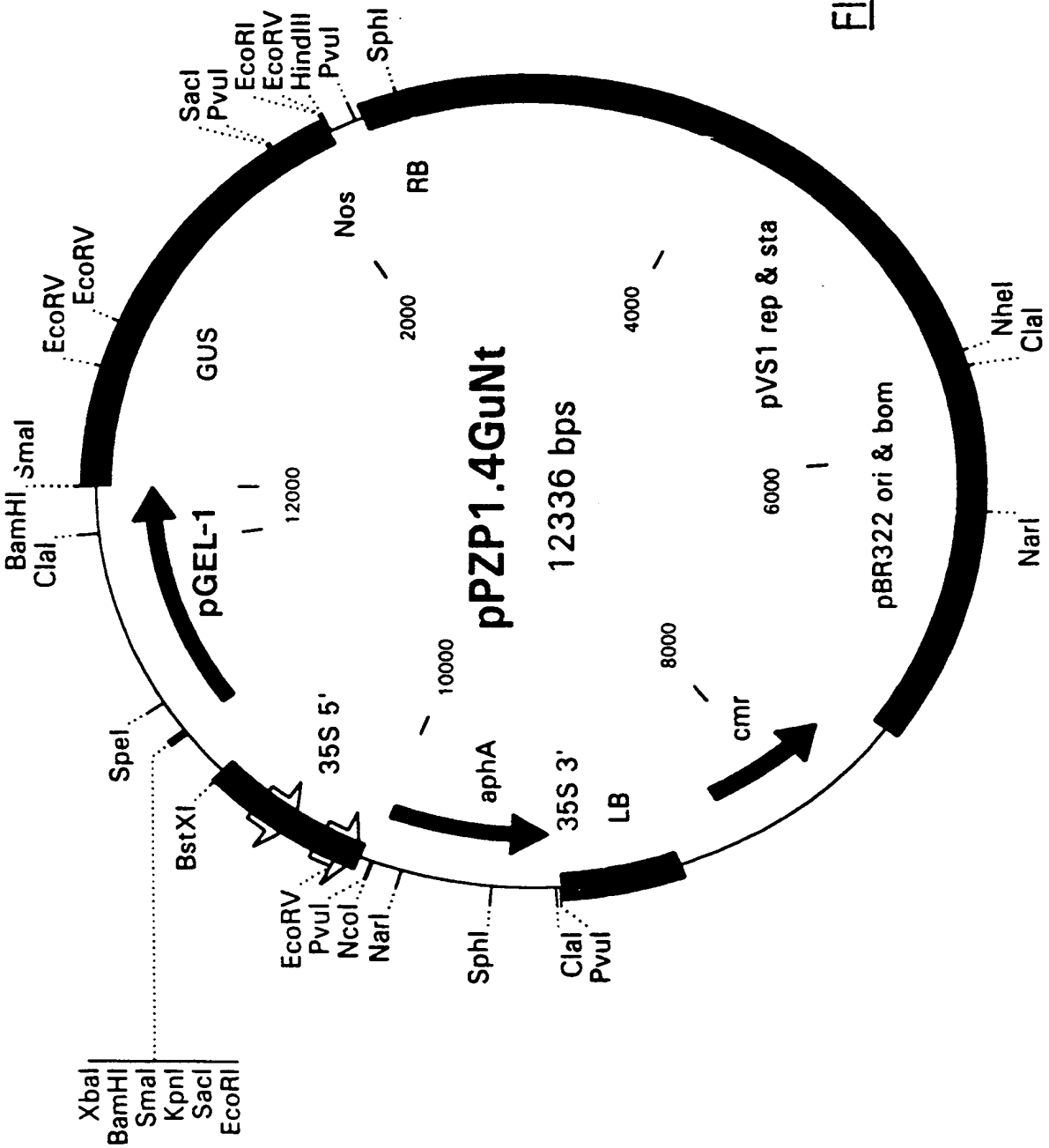
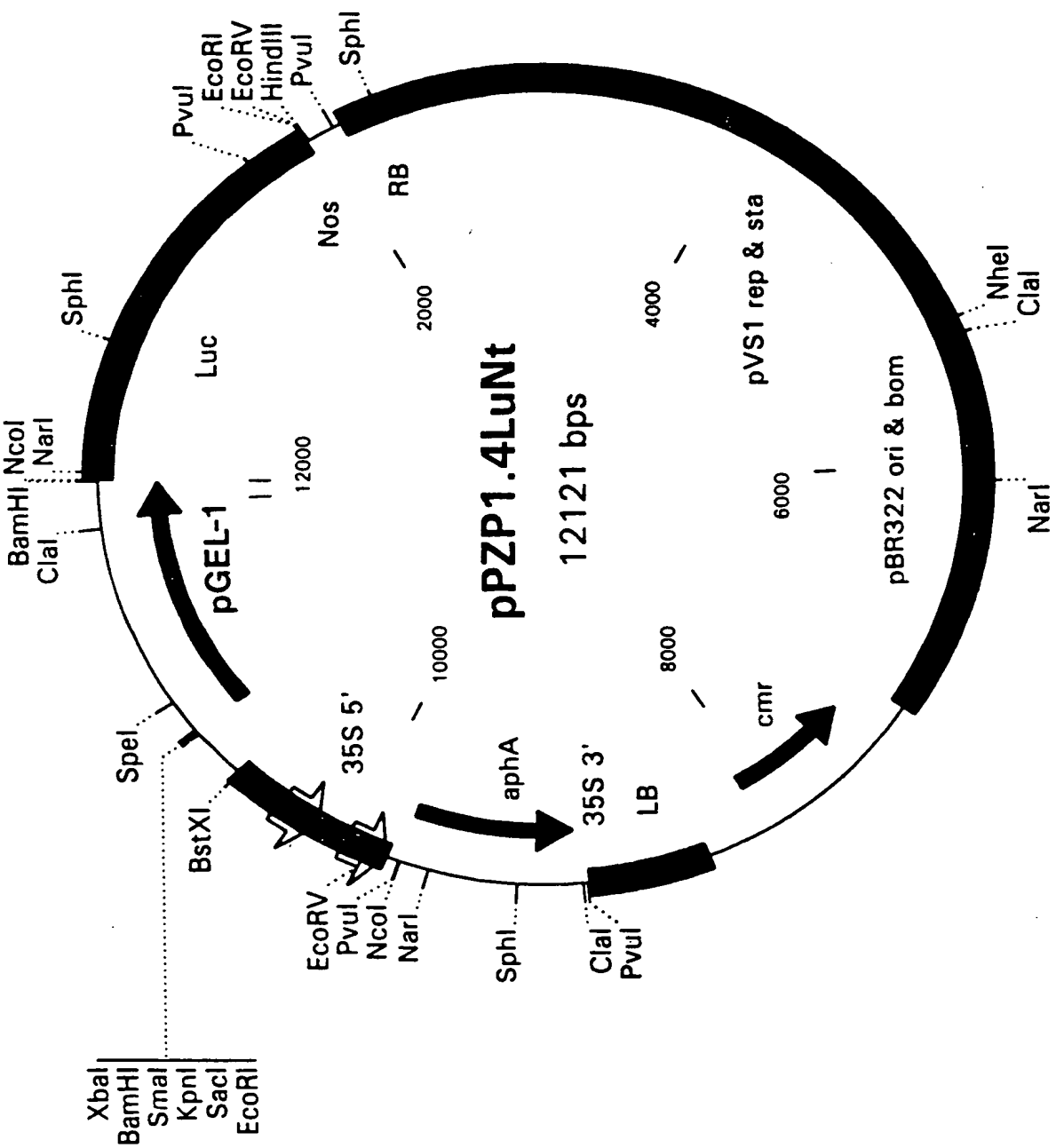


FIGURE 6A(iv)


pPZP1.4LuNt
12121 bps
pGEL-1
Luc
Nos
RB
pVS1 rep & sta
pBR322 ori & bom
aphA
cmr
35S 5'
35S 3'
LB
2000
4000
6000
8000
10000
12000
SphI
EcoRI
EcoRV
HindIII
PvuI
NheI
ClaI
NarI
NcoI
BamHI
SpeI
BstXI
XbaI
SmaI
KpnI
SacI

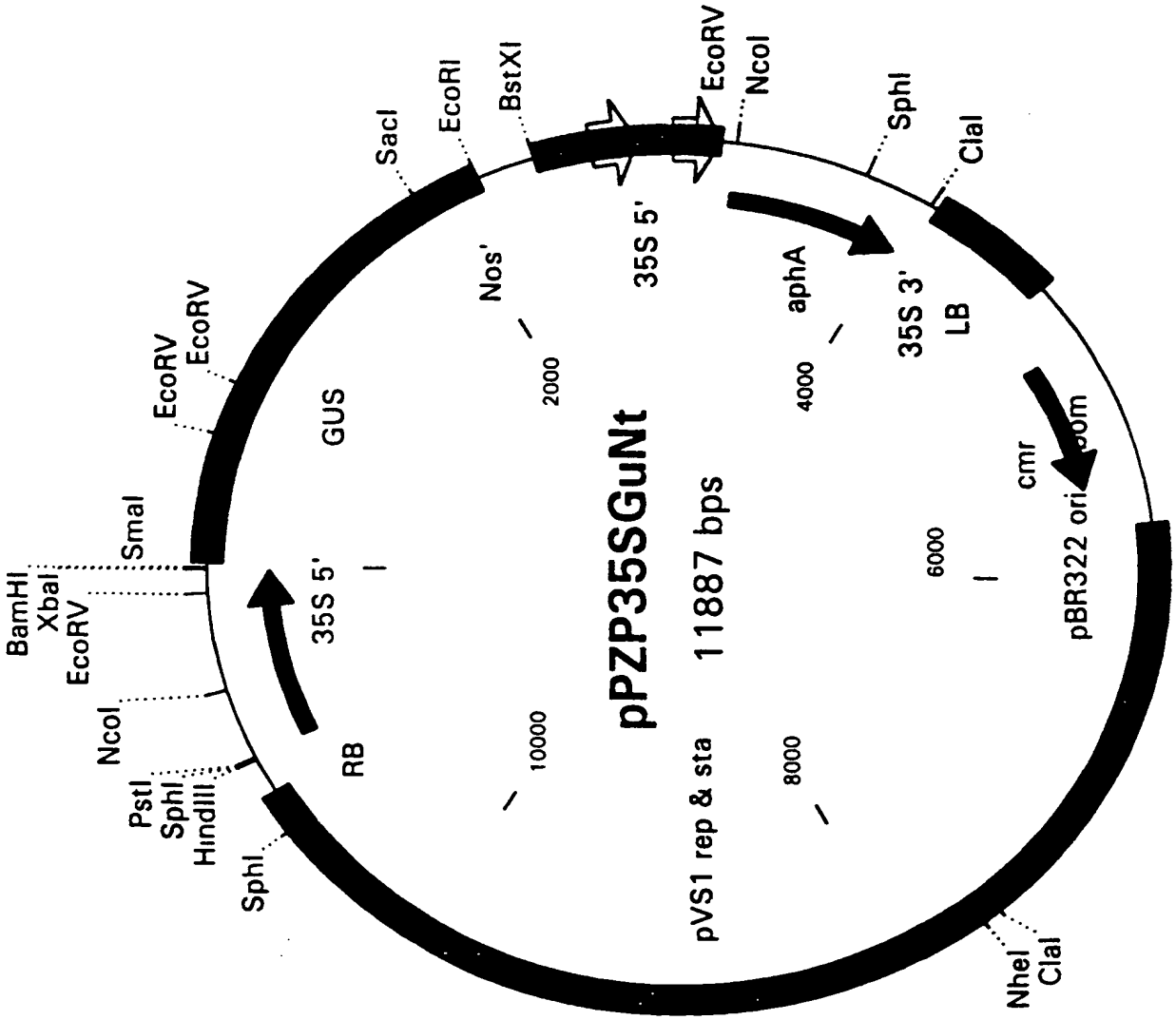

pPZP35SGuNt
11887 bps
pVS1 rep & sta
RB
35S 5'
GUS
Nos'
35S 5'
aphA
35S 3'
LB
cmr
pBR322 ori
2000
4000
6000
8000
10000
SmaI
BamHI
XbaI
EcoRV
NcoI
PstI
SphI
HindIII
SphI
EcoRV
EcoRV
SacI
EcoRI
BstXI
EcoRV
NcoI
SphI
ClaI
NheI
ClaI

FIGURE 6A(v)

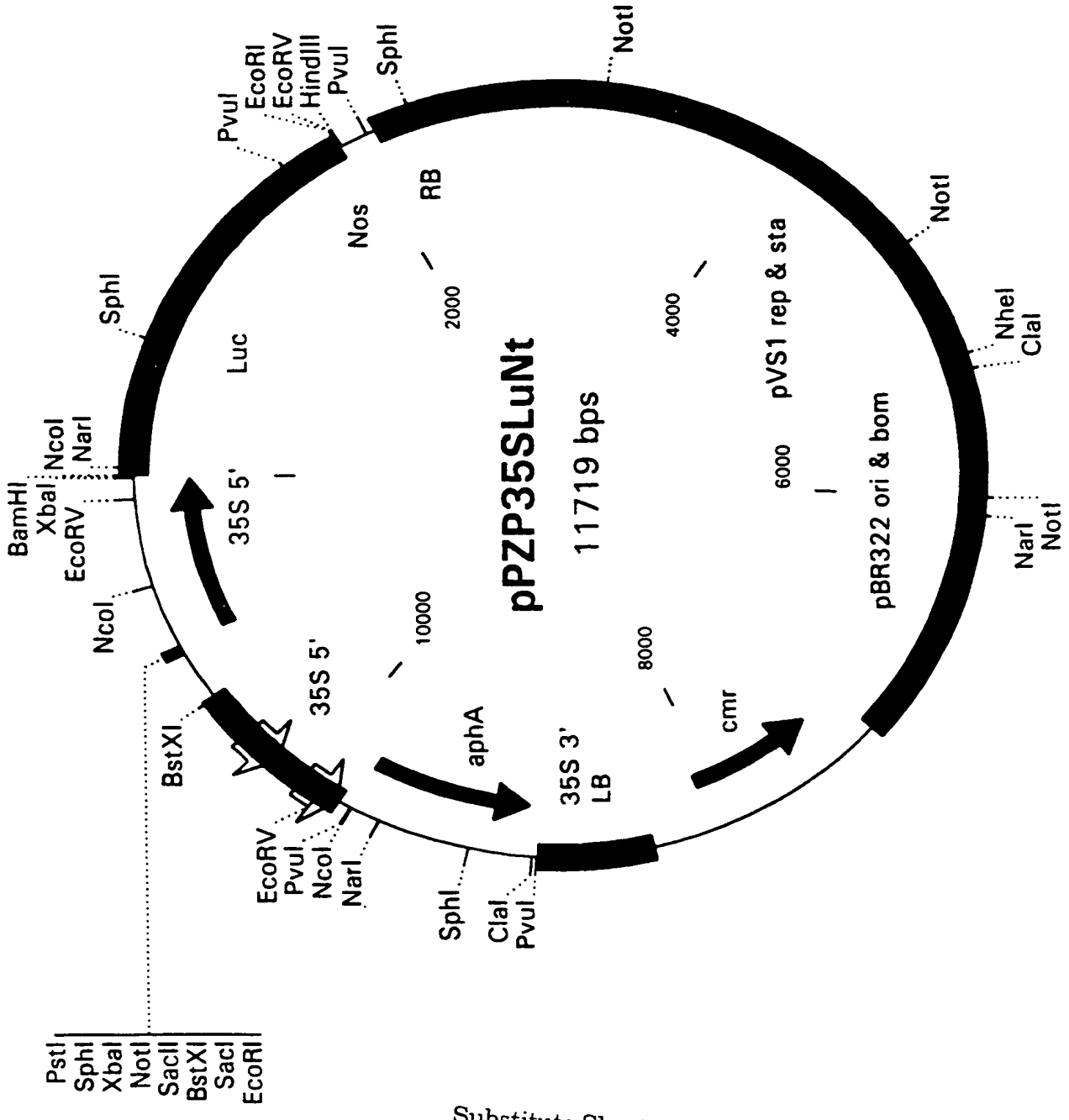

pPZP35SLuNt
11719 bps
2000
4000
6000
8000
10000
Nos
RB
pVS1 rep & sta
pBR322 ori & bom
Luc
35S 5'
35S 5'
aphA
35S 3'
LB
cmr
PstI
SphI
XbaI
NotI
SacII
BstXI
SacI
EcoRI
BamHI
XbaI
EcoRV
NcoI
NarI
SphI
PvuI
EcoRI
EcoRV
HindIII
PvuI
SphI
NotI
NotI
NheI
ClaI
NarI
NotI
BstXI
EcoRV
PvuI
NcoI
NarI
SphI
ClaI
PvuI

FIGURE 6A(vi)

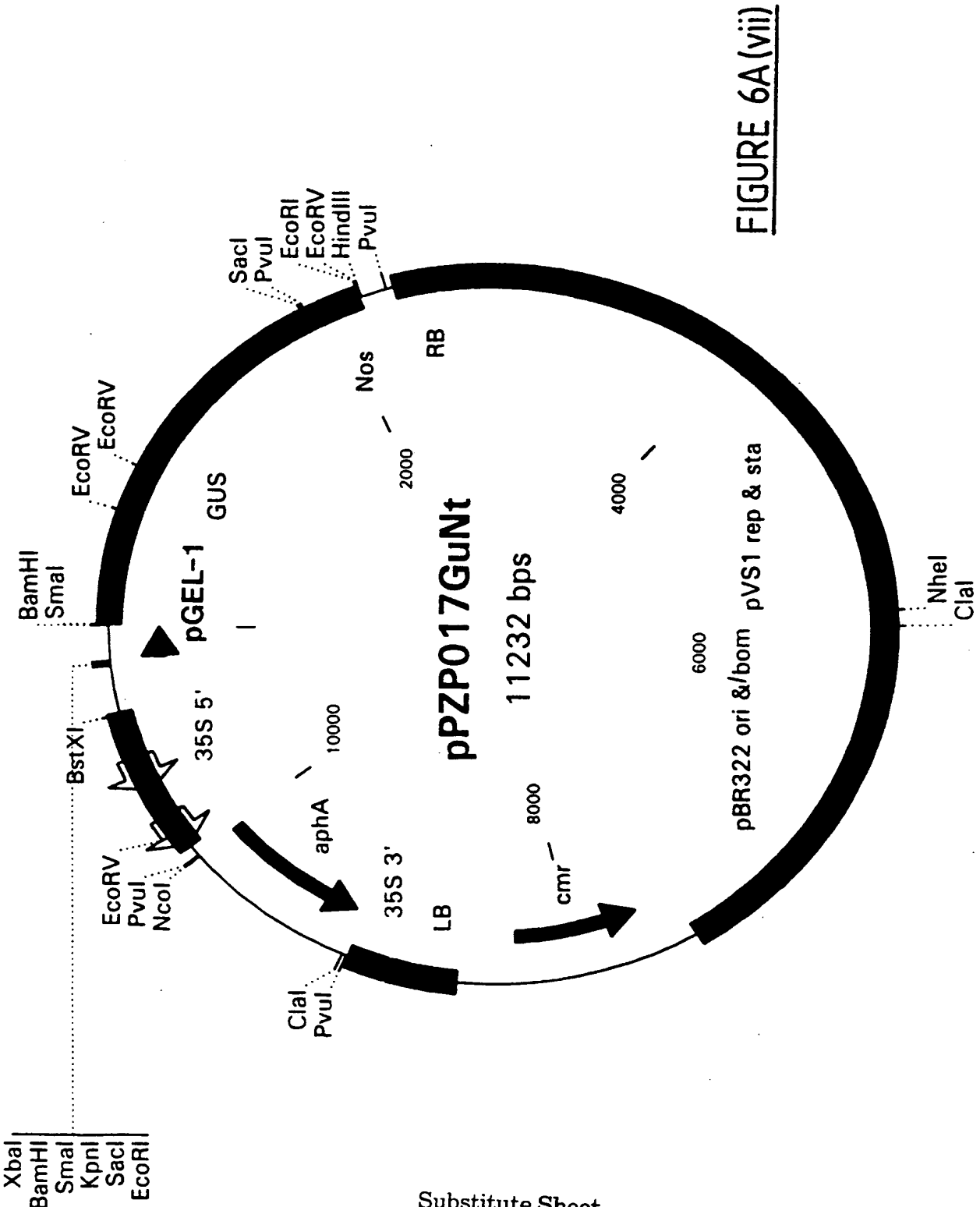


FIGURE 6A(vii)

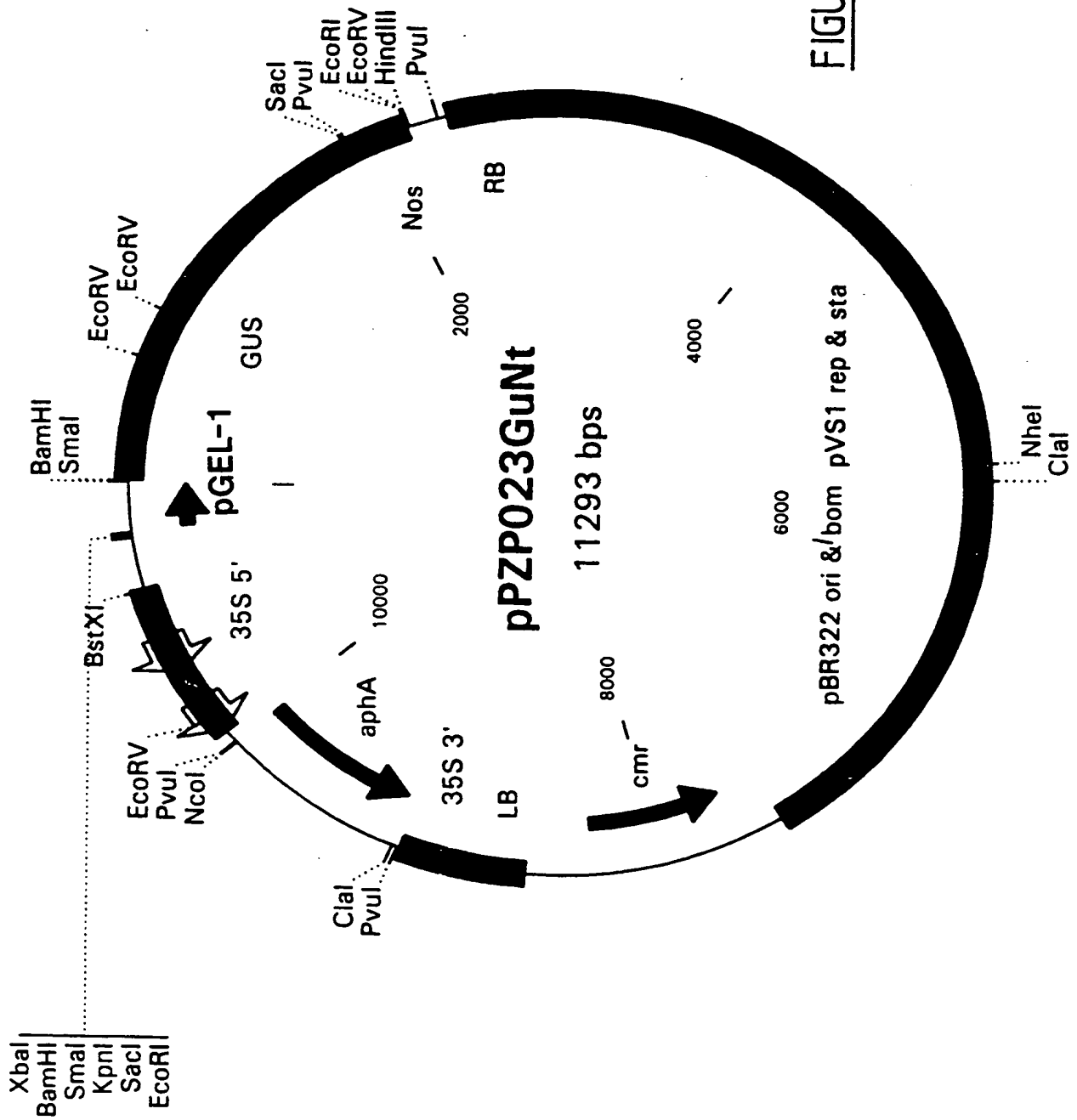


FIGURE 6A(viii)

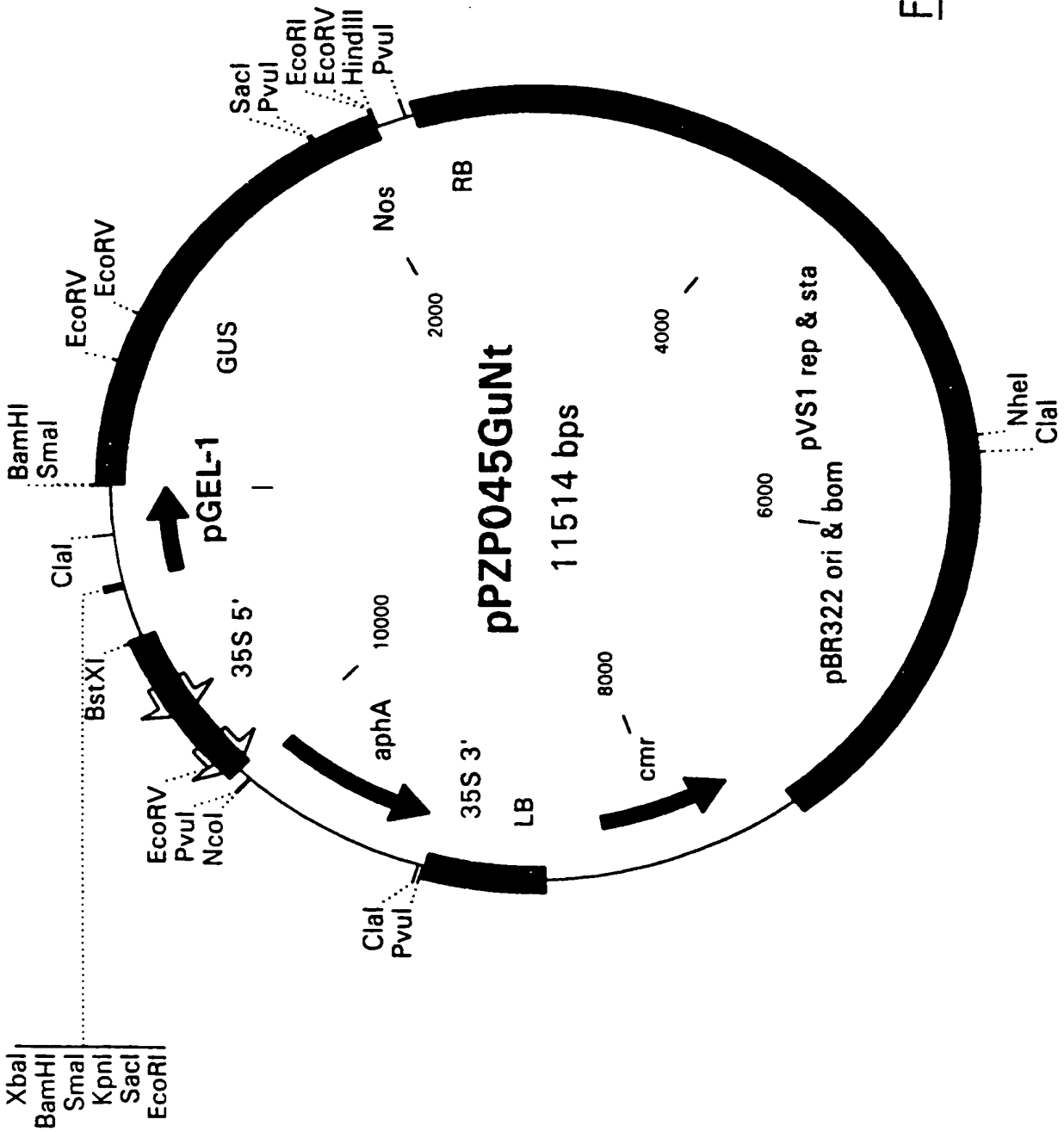

pPZP045GuNt
11514 bps
pGEL-1
GUS
35S 5'
35S 3'
aphA
LB
RB
Nos
cmr
pBR322 ori & bom
pVS1 rep & sta
2000
4000
6000
8000
10000
SacI
PvuI
EcoRI
EcoRV
HindIII
PvuI
EcoRV
EcoRV
BamHI
SmaI
ClaI
BstXI
EcoRV
PvuI
NcoI
ClaI
PvuI
NheI
ClaI
XbaI
BamHI
SmaI
KpnI
SacI
EcoRI

FIGURE 6A(ix)

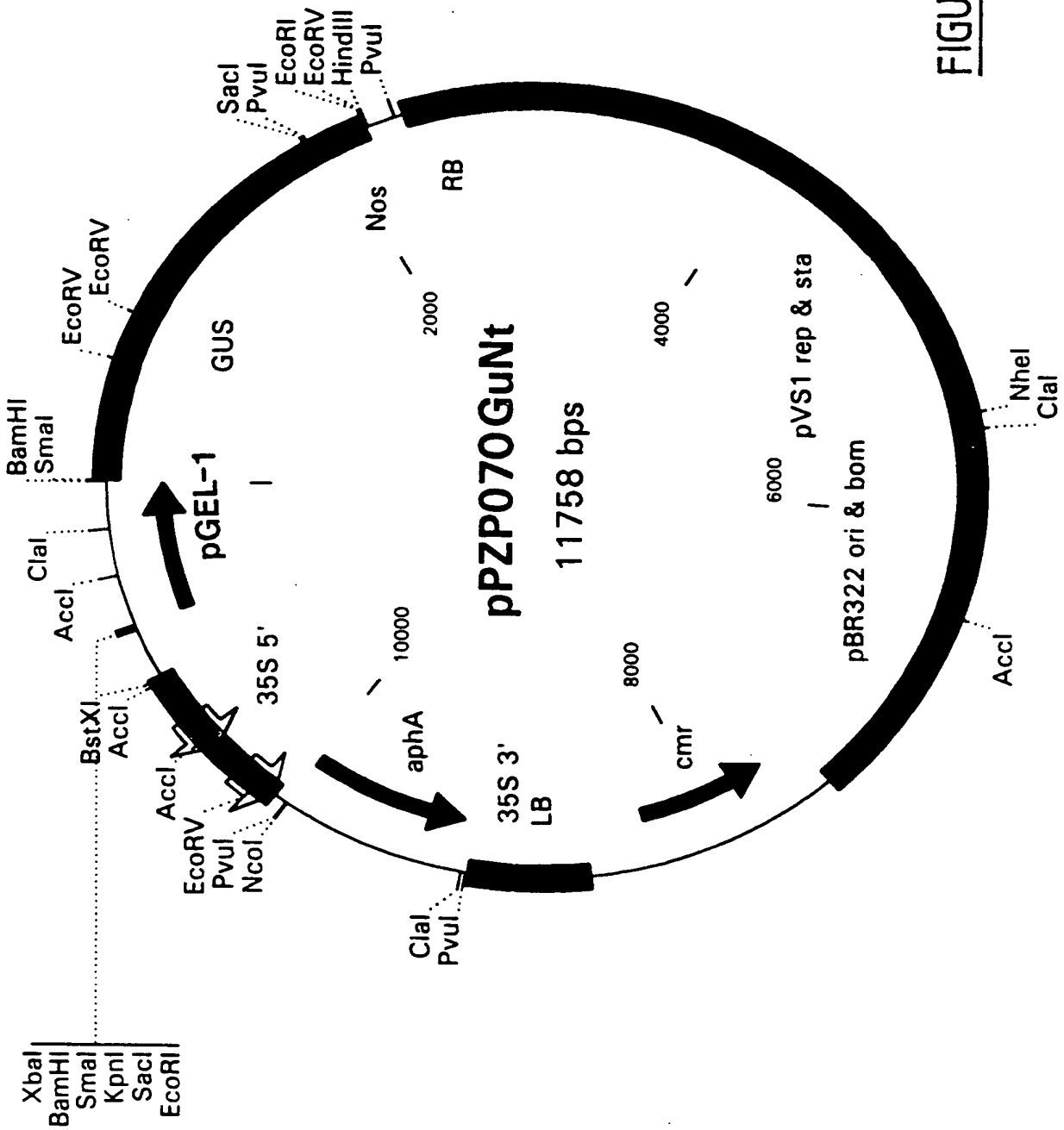

pPZP070GuNt
11758 bps
pGEL-1
GUS
35S 5'
aphA
35S 3'
LB
cmr
pBR322 ori & bom
pVS1 rep & sta
RB
Nos
2000
4000
6000
8000
10000
SacI
PvuI
EcoRI
EcoRV
HindIII
PvuI
EcoRV
EcoRV
BamHI
SmaI
ClaI
AccI
BstXI
AccI
AccI
EcoRV
PvuI
NcoI
ClaI
PvuI
NheI
ClaI
AccI
XbaI
BamHI
SmaI
KpnI
SacI
EcoRI

FIGURE 6A(x)

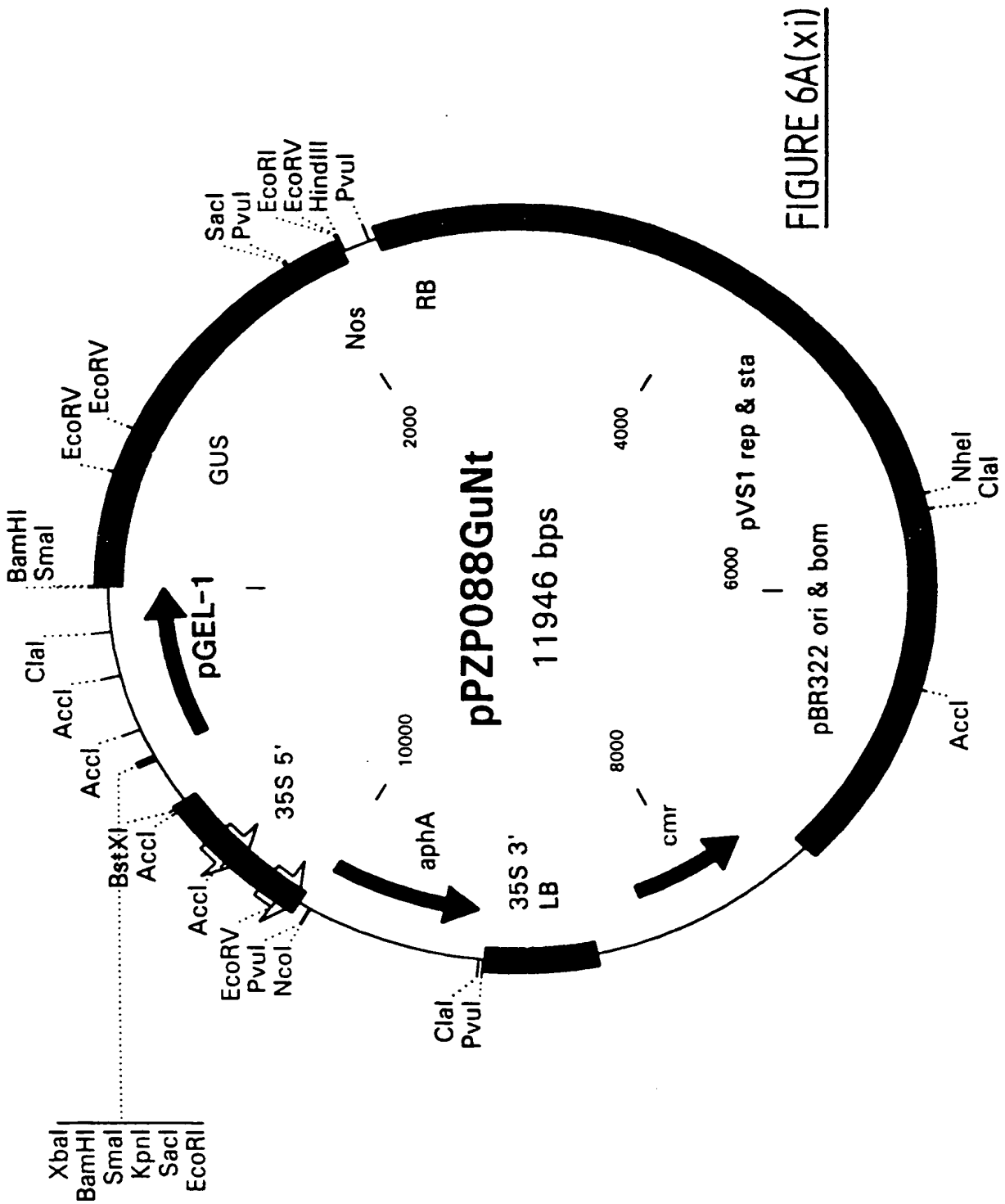

pPZP088GuNt
11946 bps
pGEL-1
GUS
35S 5'
aphA
35S 3'
LB
Nos
RB
pVS1 rep & sta
pBR322 ori & bom
cmr
2000
4000
6000
8000
10000
SacI
PvuI
EcoRI
EcoRV
HindIII
PvuI
EcoRV
EcoRV
BamHI
SmaI
ClaI
AccI
AccI
BstXI
AccI
AccI
EcoRV
PvuI
NcoI
ClaI
PvuI
NheI
ClaI
AccI
XbaI
BamHI
SmaI
KpnI
SacI
EcoRI

FIGURE 6A(xi)

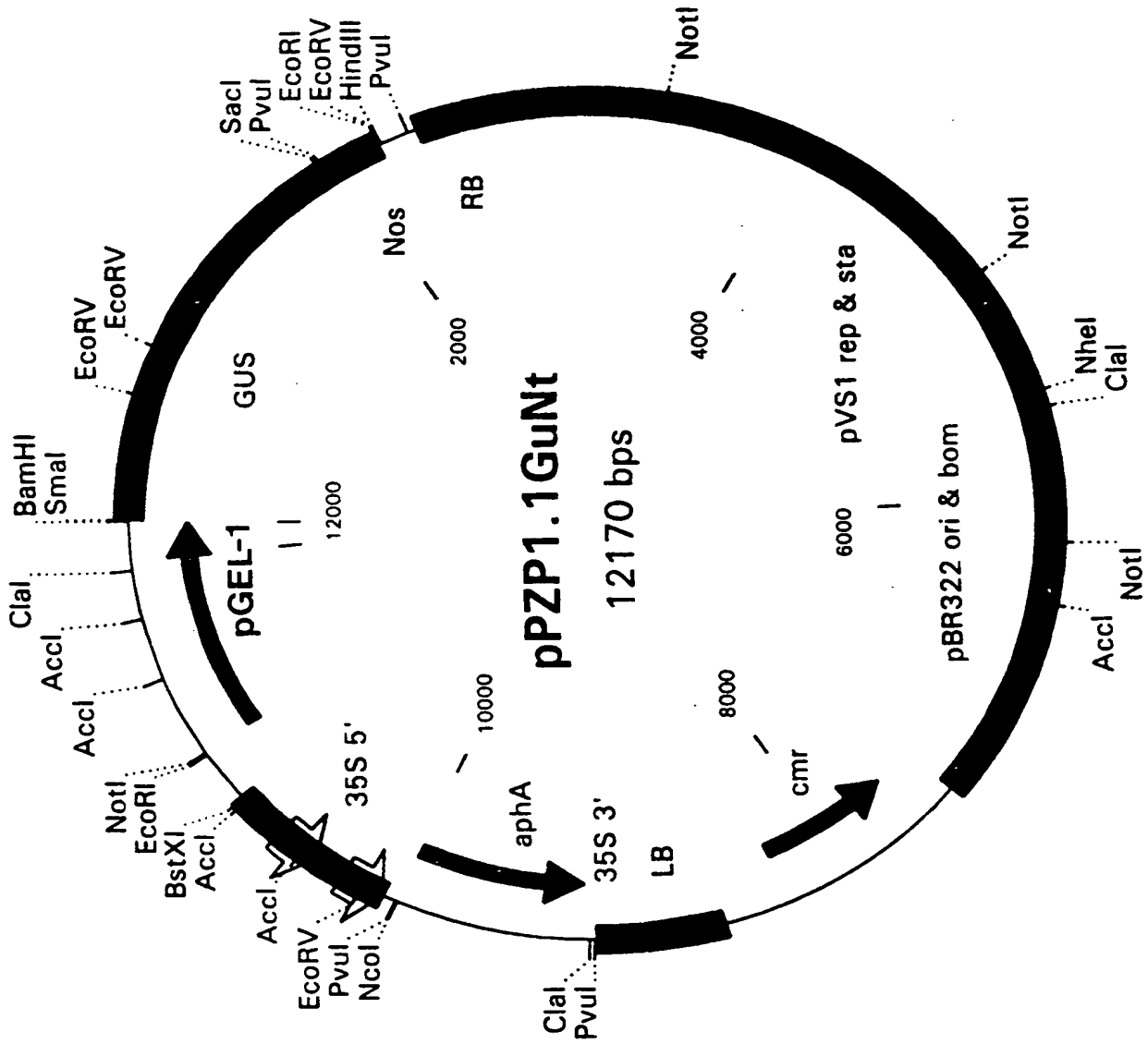
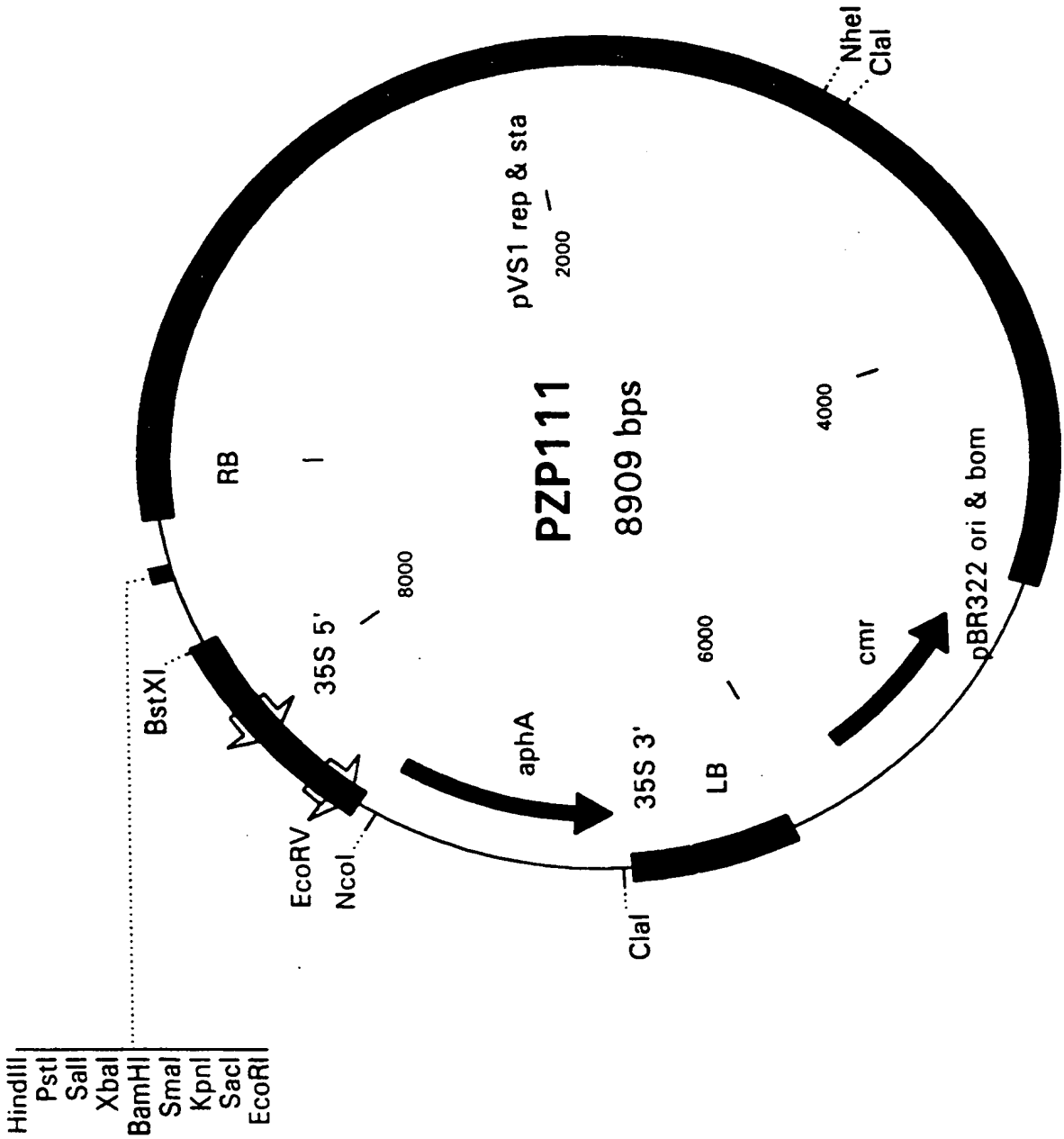


FIGURE 6A(xii)

FIGURE 6B


PZP111
8909 bps
pVS1 rep & sta
2000
4000
6000
8000
NheI
ClaI
RB
35S 5'
aphA
35S 3'
LB
cmr
pBR322 ori & bom
BstXI
EcoRV
NcoI
ClaI
HindIII
PstI
SalI
XbaI
BamHI
SmaI
KpnI
SacI
EcoRI

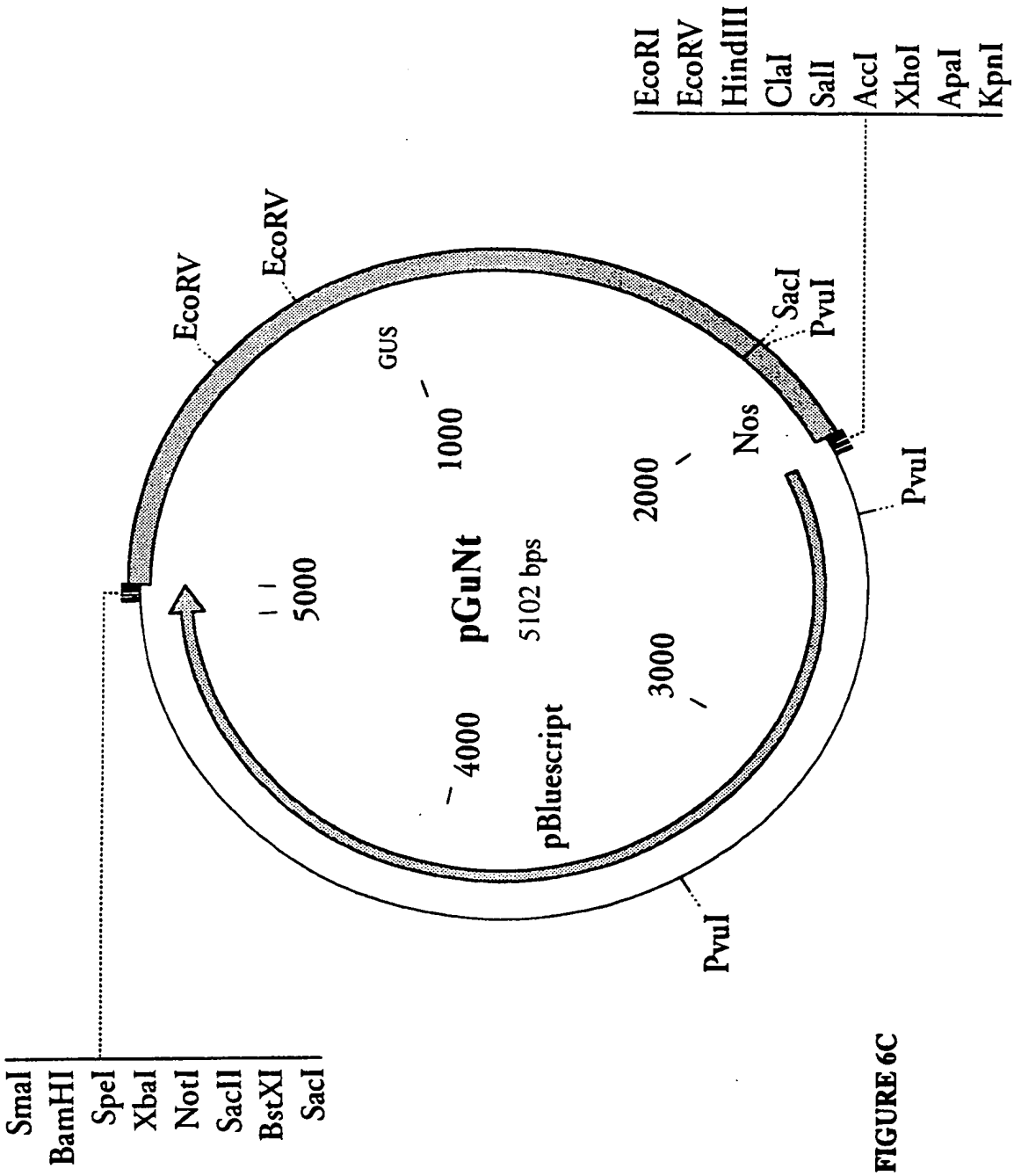

EcoRI
EcoRV
HindIII
ClaI
SalI
AccI
XhoI
ApaI
KpnI
EcoRV
EcoRV
GUS
1000
SacI
PvuI
Nos
2000
PvuI
5000
pGuNt
5102 bps
3000
4000
pBluescript
PvuI
SmaI
BamHI
SpeI
XbaI
NotI
SacII
BstXI
SacI

FIGURE 6C

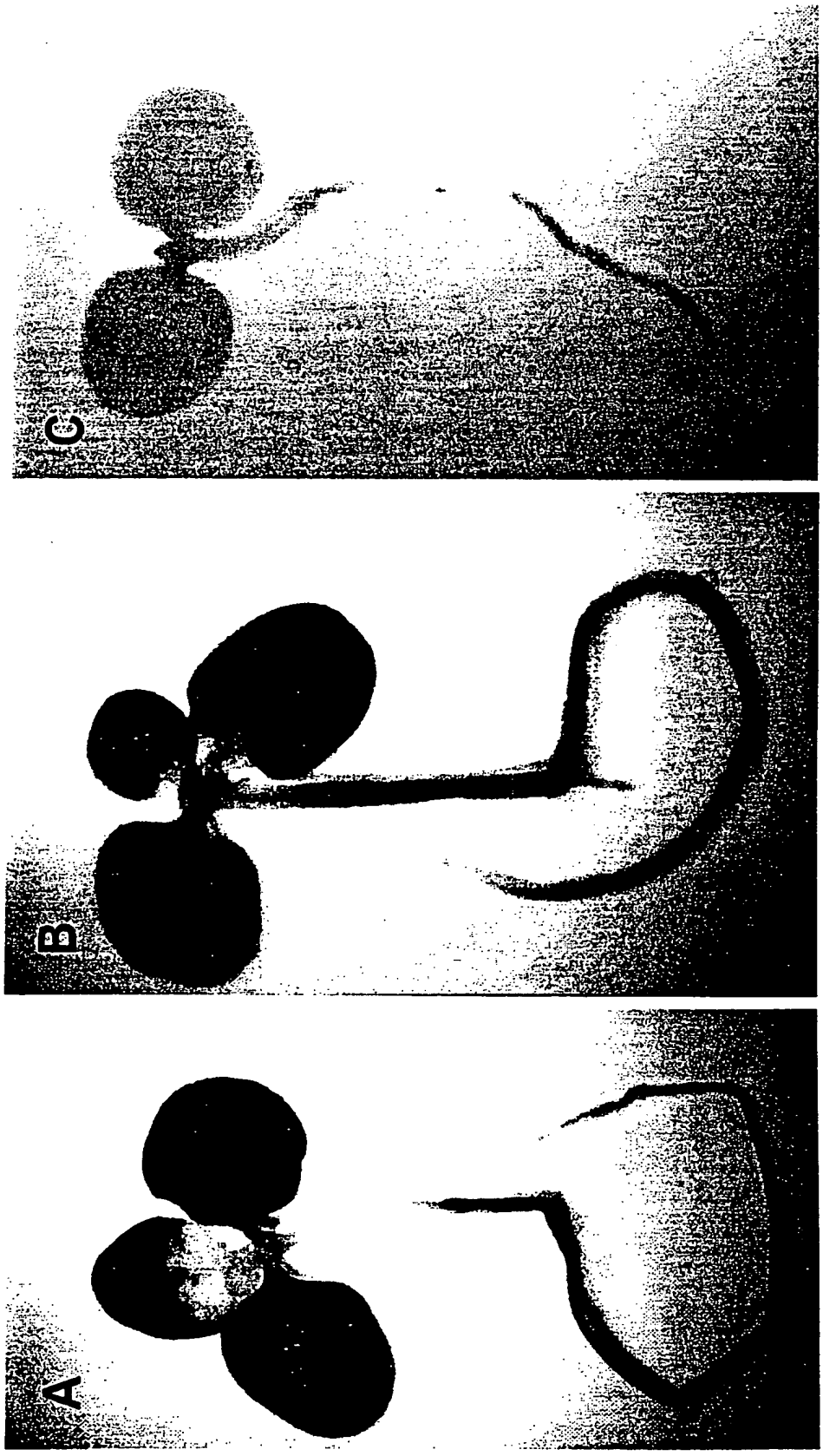


FIGURE 7

FIGURE 8B

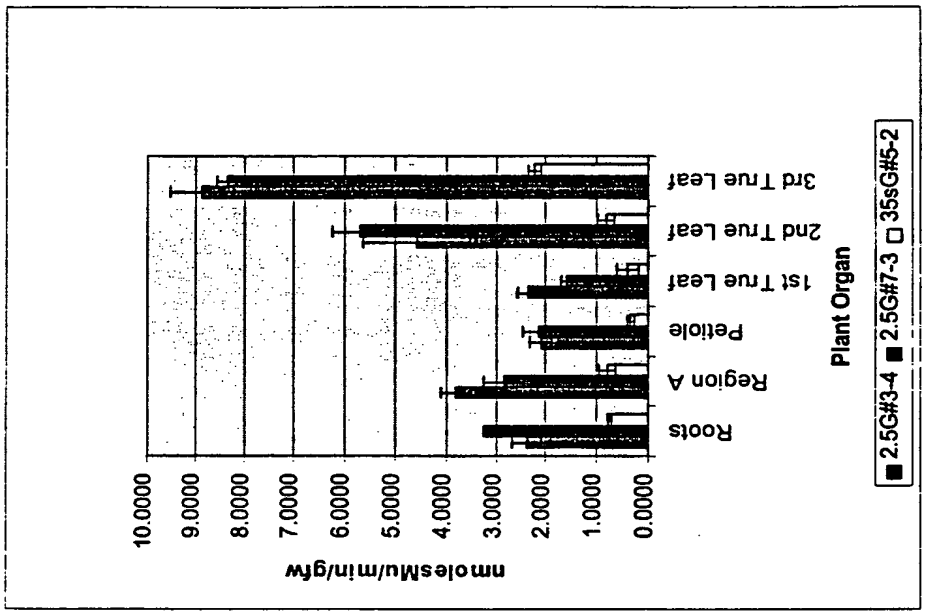


FIGURE 8A

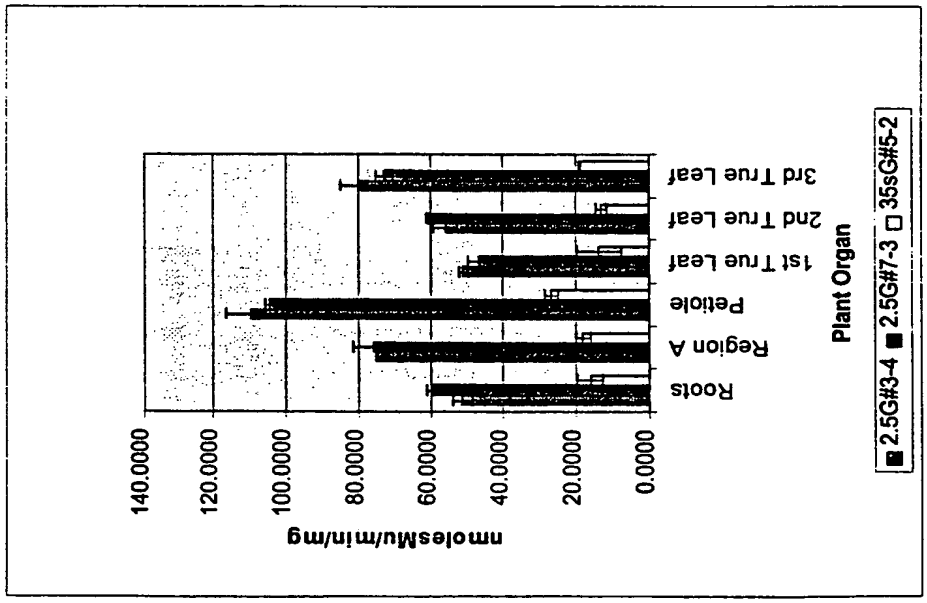


FIGURE 9B

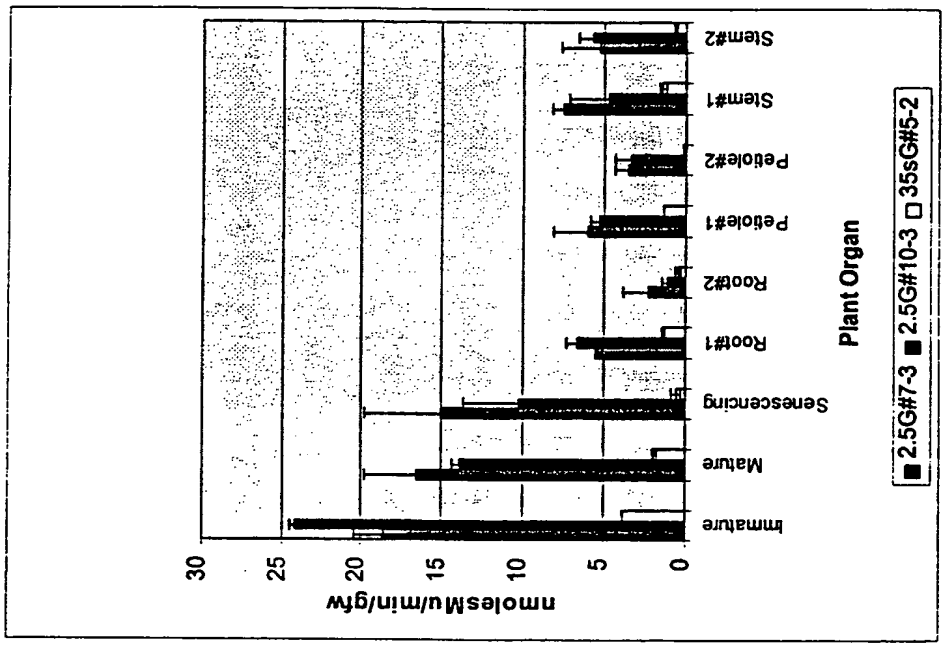


FIGURE 9A

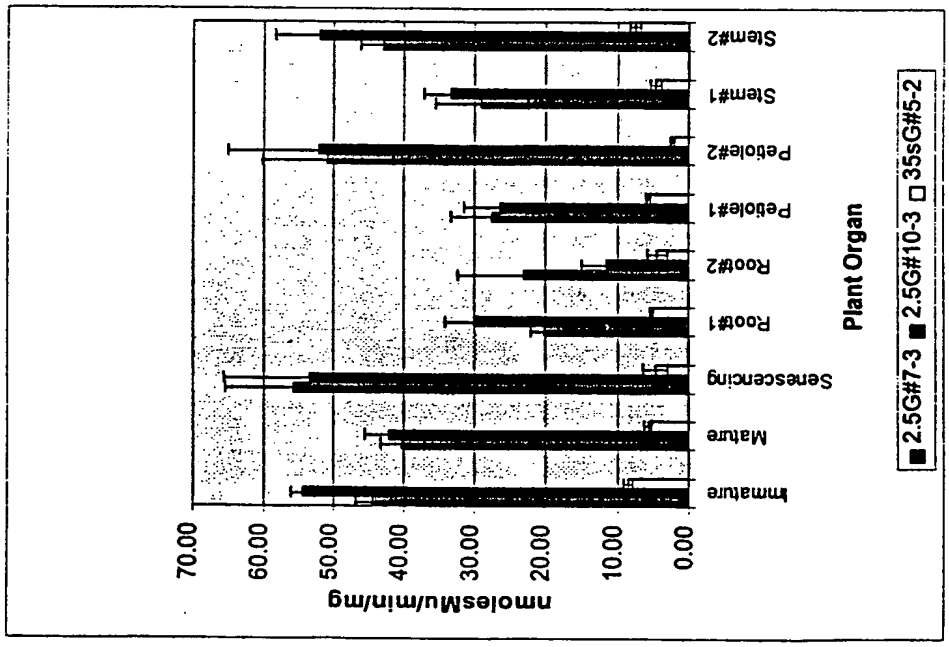


FIGURE 10B

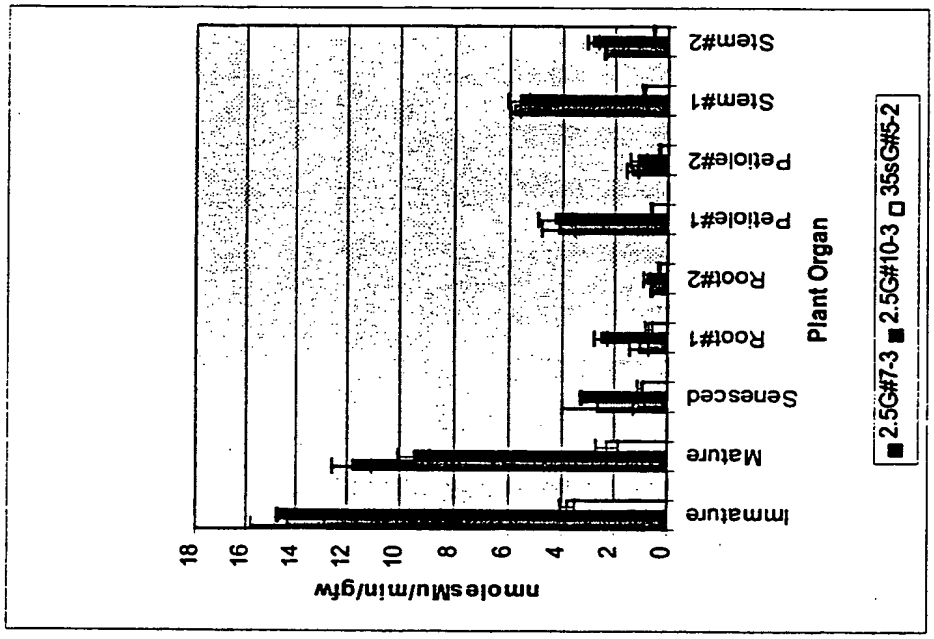
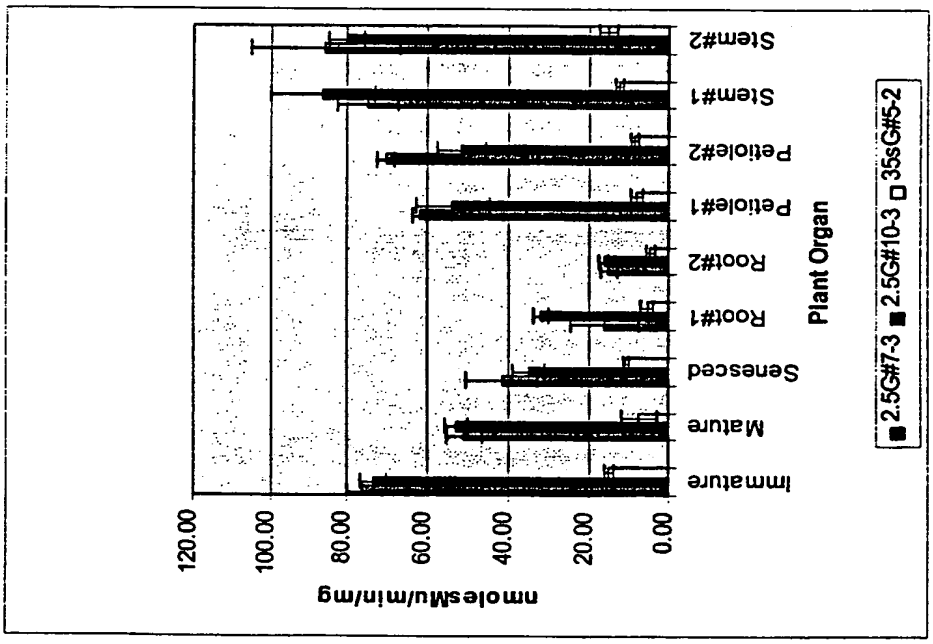


FIGURE 10A



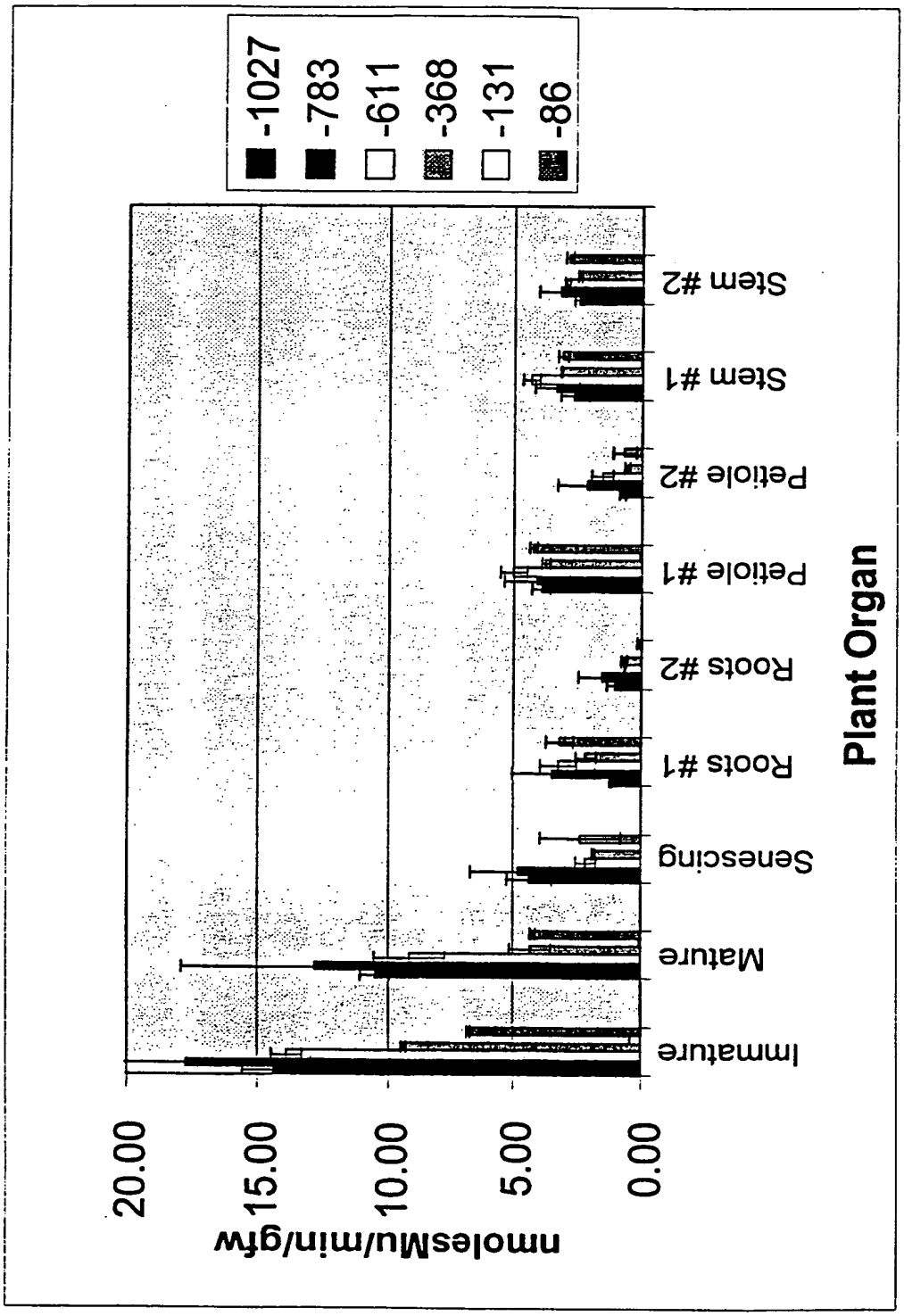
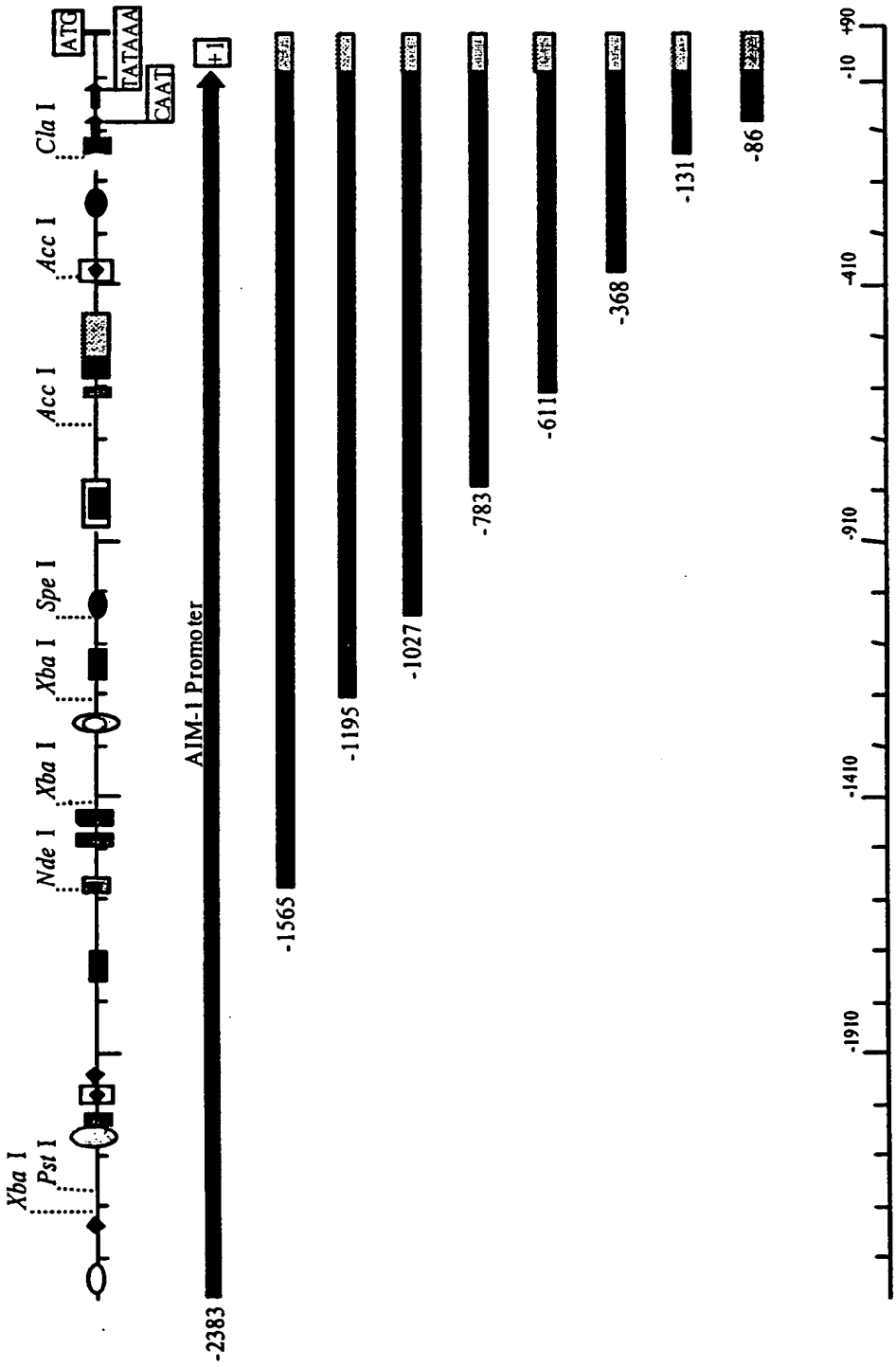

-1027
-783
-611
-368
-131
-86
Immature
Mature
Senescing
Roots #1
Roots #2
Petiole #1
Petiole #2
Stem #1
Stem #2
Plant Organ
nmolesMu/min/gfw
20.00
15.00
10.00
5.00
0.00

FIGURE 11

FIGURE 12


Xba I
Pst I
Nde I
Xba I
Xba I
Spe I
Acc I
Acc I
Acc I
Cla I
ATG
TATAAA
CAAT
+1
AIM-1 Promoter
-2383
-1565
-1195
-1027
-783
-611
-368
-131
-86
-1910
-1410
-910
-410
-10 +90

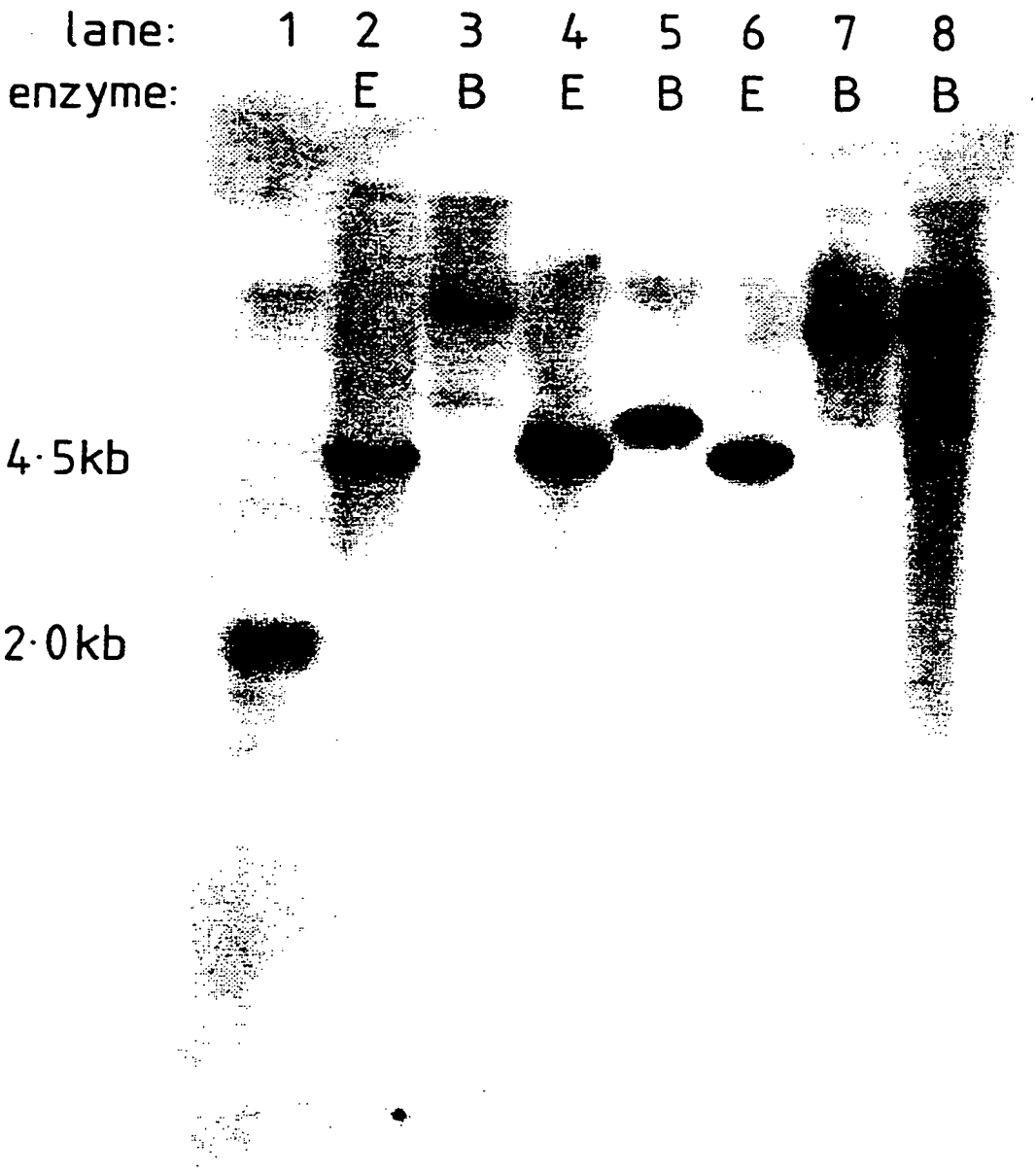


FIGURE 13

SEQUENCE LISTING

<110> THE UNIVERSITY OF QUEENSLAND

<120> A NOVEL PLANT PROMOTER AND USES THEREFOR

<130> 2209376/EJH

<140>
<141>

<150> PP5572
<151> 1998-08-31

<160> 9

<170> PatentIn Ver. 2.0

<210> 1
<211> 1923
<212> DNA
<213> Plant

<220>
<221> CDS
<222> (88)..(1539)

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actccacact ctaaccacat acaccat atg ggt ttc aag gcc atg gac caa act 114
Met Gly Phe Lys Ala Met Asp Gln Thr
1 5

ccc ttg ttg tcc aag atg gct att ggg gat gga cat ggc gaa tca tcc 162
Pro Leu Leu Ser Lys Met Ala Ile Gly Asp Gly His Gly Glu Ser Ser
10 15 20 25

cca tac ttt gat gga tgg aag gct tat gat caa aac ccc ttt cat ccc 210
Pro Tyr Phe Asp Gly Trp Lys Ala Tyr Asp Gln Asn Pro Phe His Pro
30 35 40

aca gat aat cct aac ggt gtt atg caa atg ggt ctt gct gag aat cag 258
Thr Asp Asn Pro Asn Gly Val Met Gln Met Gly Leu Ala Glu Asn Gln
45 50 55

```
ctt acc tct gat ttg gtt gaa gat tgg ata ctg aac aac cct gaa gcc   306
Leu Thr Ser Asp Leu Val Glu Asp Trp Ile Leu Asn Asn Pro Glu Ala
         60                  65                  70

tcc att tgc act cca gaa gga ata aat gat ttc agg gcc ata gct aac   354
Ser Ile Cys Thr Pro Glu Gly Ile Asn Asp Phe Arg Ala Ile Ala Asn
     75                  80                  85

ttt cag gat tat cat ggt ctg gcc gag ttc aga aat gct gtg gct aaa   402
Phe Gln Asp Tyr His Gly Leu Ala Glu Phe Arg Asn Ala Val Ala Lys
 90                  95                 100                 105

ttt atg gct aga aca agg gga aac aga atc acg ttt gac cct gac cgt   450
Phe Met Ala Arg Thr Arg Gly Asn Arg Ile Thr Phe Asp Pro Asp Arg
                110                 115                 120

att gtc atg agc ggt gga gcc acc gga gca cac gaa gtc act gcc ttt   498
Ile Val Met Ser Gly Gly Ala Thr Gly Ala His Glu Val Thr Ala Phe
            125                 130                 135

tgt ttg gca gat ccc ggc gag gca ttc tta gtg ccc att ccc tat tat   546
Cys Leu Ala Asp Pro Gly Glu Ala Phe Leu Val Pro Ile Pro Tyr Tyr
        140                 145                 150

cca ggc ttt gac cgg gat ttg agg tgg aga aca gga gtt aaa ctt gtt   594
Pro Gly Phe Asp Arg Asp Leu Arg Trp Arg Thr Gly Val Lys Leu Val
    155                 160                 165

cca gtt atg tgc gat agc tca aat aat ttc gtg ttg aca aag gaa gca   642
Pro Val Met Cys Asp Ser Ser Asn Asn Phe Val Leu Thr Lys Glu Ala
170                 175                 180                 185

ttg gaa gat gcc tat gag aaa gca aga gag gat aac atc aga gta aag   690
Leu Glu Asp Ala Tyr Glu Lys Ala Arg Glu Asp Asn Ile Arg Val Lys
                190                 195                 200

ggt tta ctg atc acc aat cca tca aat cca tta ggc aca atc atg gac   738
Gly Leu Leu Ile Thr Asn Pro Ser Asn Pro Leu Gly Thr Ile Met Asp
            205                 210                 215

aga aag aca ctg aga acc gtg gtg agc ttc atc aat gag aag cgt atc   786
Arg Lys Thr Leu Arg Thr Val Val Ser Phe Ile Asn Glu Lys Arg Ile
        220                 225                 230

cac ctt gta tgt gat gaa ata tat gct gca aca gtt ttc agc caa ccc   834
His Leu Val Cys Asp Glu Ile Tyr Ala Ala Thr Val Phe Ser Gln Pro
```

```
     235                 240                 245

ggt ttc ata agc ata gct gag ata tta gag gat gaa aca gac ata gag   882
Gly Phe Ile Ser Ile Ala Glu Ile Leu Glu Asp Glu Thr Asp Ile Glu
250                 255                 260                 265

tgt gac cgc aac ctc gta cac att gtt tat agt ctt tca aag gac atg   930
Cys Asp Arg Asn Leu Val His Ile Val Tyr Ser Leu Ser Lys Asp Met
                270                 275                 280

ggg ttc cct ggc ttc aga gtc ggc atc ata tac tct tac aat gat gct   978
Gly Phe Pro Gly Phe Arg Val Gly Ile Ile Tyr Ser Tyr Asn Asp Ala
            285                 290                 295

gtg gtt aat tgt gca cgc aaa atg tca agc ttt gga ttg gtg tca aca   1026
Val Val Asn Cys Ala Arg Lys Met Ser Ser Phe Gly Leu Val Ser Thr
        300                 305                 310

cag act cag tat ctt tta gca tcg atg cta aat gat gat gag ttt gtg   1074
Gln Thr Gln Tyr Leu Leu Ala Ser Met Leu Asn Asp Asp Glu Phe Val
    315                 320                 325

gag agg ttt ctg gca gag agt gca aag agg ttg gct caa agg ttc agg   1122
Glu Arg Phe Leu Ala Glu Ser Ala Lys Arg Leu Ala Gln Arg Phe Arg
330                 335                 340                 345

gtt ttc act ggg ggg ttg gcc aaa gtt ggc ata aag tgc ttg caa agc   1170
Val Phe Thr Gly Gly Leu Ala Lys Val Gly Ile Lys Cys Leu Gln Ser
                350                 355                 360

aat gct ggt cta ttt gtg tgg atg gat tta agg caa ctt ctc aaa aag   1218
Asn Ala Gly Leu Phe Val Trp Met Asp Leu Arg Gln Leu Leu Lys Lys
            365                 370                 375

cca act ttc gac tct gaa acg gag ctt tgg aaa gtt atc att cat gaa   1266
Pro Thr Phe Asp Ser Glu Thr Glu Leu Trp Lys Val Ile Ile His Glu
        380                 385                 390

gtt aag atc aat gtt tca cct ggc tat tcc ttc cat tgc act gag cca   1314
Val Lys Ile Asn Val Ser Pro Gly Tyr Ser Phe His Cys Thr Glu Pro
    395                 400                 405

ggg tgg ttt agg gtg tgc tat gcc aac atg gat gat atg gct gtg caa   1362
Gly Trp Phe Arg Val Cys Tyr Ala Asn Met Asp Asp Met Ala Val Gln
410                 415                 420                 425
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gtg gtg tct aat aag aaa cat tgt tgg cac agt aac ttg agg ctg agc   1458
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Ile Asn Asp Phe Arg Ala Ile Ala Asn Phe Gln Asp Tyr His Gly Leu
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Arg Trp Arg Thr Gly Val Lys Leu Val Pro Val Met Cys Asp Ser Ser
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Ser Asn Pro Leu Gly Thr Ile Met Asp Arg Lys Thr Leu Arg Thr Val
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INTERNATIONAL SEARCH REPORT

Internati nal application No.
PCT/AU 99/00705

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C12N 15/29

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
SEE BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
see below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EMBL:SEQ ID NOS. 1-9
ORBIT (WPAT): C12N - 015/IC and A01H/IC and ACC SYNTHASE OR AMINOCYCLOPROPANE OR ETHYLENE
STN (Medline: dgene) : promoter and gene expression regulation/CT; SEQ. ID. No 2 (inpart).

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,Y	Plant Molecular Biology, Vol. 18, pp 793-797 (1992). Botella et al. See whole document	1-21
X,Y	Plant Molecular Biology, Vol. 20, pp425-436 (1992). Botella et al. p429-p430	1-21
X,Y	Proc. Natl. Acad. Sci. USA, vol. 92, pp1595-1598 (1995) Botella et al. p1597	1-21
P,X	Plant Cell Physiol, 40(4), pp 431-438 (1999). Yoon et al. See whole document	1-21

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" Document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
	27 OCT 1999

Name and mailing address of the ISA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No.: (02) 6285 3929	MADHU K. JOGIA Telephone No.: (02) 6283 2512

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 99/00705

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMBL Accession No. X67100 Liu et al.	1-3, 6, 7
X	Plant Journal, 14(5), pp 573-581. Peck et al. (June 1998) Fig 4; p 577	1-3
X	Plant Molecular Biology, 28(2), pp 293-301 (1995) Peck et al	1-3,8
X	US 5523221 (Weiner , M.P.) published June, 1996. See seq. 1,2 and 3	1-3,8
X	US 5750667 (Wickens et al). published May, 1998 See sequence 7.	1-3, 9
X	US 5756343 (Wu et al) published May, 1998. See sequence 33	1-3, 9
X,Y	WO A 9806852 (University of Hawaii). published 19 Feb 1998	1-21
X,Y	US 5767376 (Stiles et al). published June, 1995.	1-4
X,Y	US 5702933 (Klee et al). published Dec., 1997.	1-4
X	WO A 9814465 (Colorado State University) published April 1998.	1-4
X	US 5723766 (Theologis et al) published June, 1995	1-4
P,X	WO A 9845445 (The Min. of Agriculture et al). published 15.10.98; pp 1-5.	1-21
X	WO A 9711166 (Botella et al) published 27.03.97; p 1-5; claims 1-17	1-21
X	WO A 9635792 (Allrad No. 1 Pty Ltd et al) published 14.11.96. See whole document.	1-21
X	WO A 9727308 (Agritope Inc.et al). published 31.07.91. See whole document.	1-21

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU 99/00705

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
US	5523221						
US	5750667	AU	39597/95	EP	765403	US	5610015
		WO	9629429	US	5677131		
US	5756343	AU	90723/91	CA	2096975	WO	9209617
WO	9806852	AU	40629/97	CZ	9900450	EP	918869
		NO	990508	US	5874269	US	5767376
US	5767376	US	5874269	AU	40629/97	CZ	9900450
		EP	918869	NO	990508	WO	9806852
US	5702933	AU	91137/91	BR	9107191	CA	2096637
		EP	564524	FI	932960	JP	9238689
		NO	923343	WO	9212249	US	5512466
WO	9814465	AU	48929/97	US	5824875		
US	5723766	AU	85114/97	CA	2091243	EP	548164
		MX	9100993	WO	9204456		
WO	9845445	AU	69273/98	ZA	9803007		
WO	9711166	AU	69200/96	EP	854916		
WO	9635792	AU	54930/96	EP	824591		
WO	9727308	AU	17559/97	AU	18466/97	CA	2243850
		CA	2243969	EP	877813	US	5783393
		US	5783394	US	5929302		

END OF ANNEX